Next Issue.—Special Dairy Cattle Number.

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JUNE, 1934.

CONTENTS.

	PAGE
EDITORIAL—	
Special Cattle Diseases Number	... 221
Honour to Capt. Adrian Jones, M.V.O., M.R.C.V.S.	... 221
OBITUARY—	
Major Harry Peele, F.R.C.V.S.	... 222
GENERAL ARTICLES—	
"Cripples" in Lambs—II. By S. E. Piercy, B.Sc.	... 223
Studies on the So-called Electrical Stunning of Animals. By. J. Roos and S. Koopmans	... 232
Observations on Active Immunisation Against Anthrax. By F. H. Manley, B.V.Sc., M.R.C.V.S.	... 245
ABSTRACTS OF CURRENT LITERATURE	... 262
CLINICAL ARTICLES—	
Deaths in Poultry Occasioned by Bee Stings. By H. P. Hamilton, B.V.Sc.(Toronto)	... 266
CORRESPONDENCE	... 266
NEWS	... 267
PERSONAL	... 268

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JUNE, 1934.

Editorial

SPECIAL CATTLE DISEASES NUMBER.

THE issue of a Special Cattle Diseases Number which had been arranged for the month of June has been carried over until July, when a very varied selection of treatises upon matters connected with dairy cattle and beef production will be introduced. This new feature of THE VETERINARY JOURNAL in introducing numbers on special subjects at frequent intervals has been much appreciated, and is forming a further *liaison* between the profession and the stockowner. We ask our readers to support the movement, as it is by collaboration of this kind that the profession itself will obtain considerable benefit and make material progress.

HONOUR TO CAPT. ADRIAN JONES, M.V.O., M.R.C.V.S.

THE Honorary Associateship of the Royal College of Veterinary Surgeons is one of the highest honours which the Council has in its power to bestow; and that the selection should on this occasion have fallen upon such a distinguished *confrère* as Capt. Adrian Jones will be approved with great sincerity by every member of the profession. It is not so generally known as it ought to be that the distinguished sculptor of those majestic works of art, the Quadriga on Constitution Hill and the Cavalry War Memorial at Stanhope Gate, is a member of the veterinary profession; although when studied, even for a few moments, by the most casual observer, it is readily obvious that the accuracy of the form and musculature

of the equine subjects bears testimony to the fact that the artist has a knowledge of animal anatomy which betokens something more than an ordinary artistic study. Although over ninety years of age, Adrian Jones still takes the greatest interest in the progress of each of the professions of which he is so distinguished a member. That he may be spared for many years to come is the wish of every reader of THE VETERINARY JOURNAL.

Obituary

MAJOR HARRY PEELE, F.R.C.V.S.

BY the death of Major Harry Peele at the age of sixty-one, Durham County and Durham City lose a very much respected citizen, and the members of the veterinary profession a brilliant colleague. Succeeding to a very large practice on the death of his father, Major Peele had not only the confidence of a very large personal *clientèle*, but, in addition, that of numerous official bodies of Durham City and County. During the later years of his life he handed over the general practice to his son, Mr. John Peele, retaining for his own personal attention the public appointments.

A devoted Territorial, he was awarded the Officers' Decoration and until some five years ago held the appointment of Director of Veterinary Services in the Northern Command. He was a regular and active attendant at the meetings of the northern branch of the National Veterinary Medical Association, in the management of which he always took a very prominent part. The sympathy of the profession will be extended to his widow and family, of which there are three sons and two daughters.

General Articles

"CRIPPLES" IN LAMBS.—II.

By S. E. PIERCY, B.Sc.

Veterinary Laboratory, Armstrong College, Newcastle-upon-Tyne.

Introduction.

In a previous article[1] an account was given of an attempt to prevent the occurrence of the condition in lambs known in the north of England as "cripples." The suggestion had been made by Stewart[2] that the disease in lambs might be due to mineral insufficiency of the food of the ewes. To test that hypothesis a supplementary ration, consisting of a specially prepared cake rich in minerals and cod-liver oil, was fed to a selected number of pregnant ewes in a flock grazing on a "cripples" farm. Conversely, an attempt was made to reproduce the disease in lambs by feeding pregnant ewes in pens on known rations containing various amounts and proportions of minerals. The results of the field experiments recorded in the paper referred to[1] indicated that a mineral supplement did not influence the incidence of "cripples," and hence that the disease could not be attributed to mineral deficiency. The results of the cognate experiment are now presented.

Experimental Details.

In the autumn of 1932 a batch of healthy five-year-old Blackface ewes was transferred from an upland farm to Cockle Park, the Northumberland County Experimental Farm, where they were allowed to graze on rough pasture and mated with a Border-Leicester ram. About Christmas, twenty-five ewes were divided into five groups of five, housed in pens provided with concrete floors, and placed on a ration of maize and poor-quality hay to accustom them to the experimental conditions in view. This ration was replaced by the experimental rations as fast as the ewes could be persuaded to consume them, and by the end of January all the animals were on diets of completely controlled mineral content.

The Diets.—The experimental rations were made up by adding known amounts of mineral salts to a basal ration low in minerals, according to the scheme outlined below. The foods chosen were maize starch, blood meal and hay, of percentage composition as shown in Table I.

TABLE I.—PERCENTAGE COMPOSITION OF COMPONENTS OF BASAL RATION.

Food	Moisture	Protein	Oil	Carbo-hydrates	Fibre	Total Ash	CaO	P_2O_5
Maize starch ...	12.38	0.08	0.50	84 70	2.10	0.24	0.02	0.06
Blood meal ...	9.06	87.10	0.14	Trace	Trace	3.00	0.05	0.20
Hay	12.23	9.25	1.25	37.10	33.23	6.94	0.75	0 36

The intention was to limit the consumption of hay in order to secure a basal ration as low as possible in minerals, but it was found impracticable to cut down the hay allowance below two-thirds of a pound per head per day. The maize and blood meal were mixed in the proportions of four to one, and fed at the rate of one pound per head per day together with five grammes of salt. Rain-water was used for drinking purposes in order to eliminate the experimental error introduced by hard tap-water.

Calculating from the analyses given, the basal ration would supply approximately 2.33 gm. CaO and 1.49 gm. P_2O_5, and assuming complete consumption of the ration these would be the maximum quantities ingested per ewe per day. Considering the grazing habits of Blackface ewes, it may be assumed that their intake would correspond to about three pounds of dry matter per head per day if they were on their natural pasture.[3] The intake of calcium and phosphorus from such grazing[4] would therefore be about 8 gm. CaO and 8 gm. P_2O_5. It will be observed, then, that the basal ration only supplied between a quarter and a third of the amount of calcium and less than one-fifth of the amount of phosphorus that would be supplied by ordinary grazing. This degree of deficiency was considered sufficient for the experimental purpose in view.

One pen of five sheep was fed on the basal ration alone, a second received a supplement of calcium, a third a supplement of phosphorus, a fourth received a supplement of both calcium and phosphorus, and a fifth received cod-liver oil to improve the vitamin content of the basal ration. The calcium supplement used was calcium carbonate, whilst the phosphorus supplement was a mixture of equal parts of di-sodium and di-potassium hydrogen phosphate. The cod-liver oil used was of guaranteed potency. The details of each ration are shown in Table II.

TABLE II.—DETAILS OF RATIONS.

TOTAL MINERAL CONTENT IN GRAMMES PER HEAD PER DAY.

	Pen I	Pen II	Pen III	Pen IV	Pen V
	Low P, High Ca.	Low Ca, High P.	Low Ca, Low P.	C.L.O. Control.	High Ca, High P.
	Basal + 10 gm. $CaCO_3$	Basal +7.5 gm. Na_2HPO_4 +7.5 gm. K_2HPO_4	Basal	Basal + 1 fluid oz. cod-liver oil	Basal +7.5 gm. Na_2HPO_4 +7.5 gm. K_2HPO_4 +10 gm. $CaCO_3$
CaO	7.93	2.33	2.33	2.33	7.93
P_2O_5	1.49	8.30	1.49	1.49	8.33
$CaO : P_2O_5$	1 : 0.19	1 : 3.56	1 : 0.64	1 : 0.64	1 : 1.05

From this table it will be noted that apart from the actual mineral intake per day, the ratio of calcium to phosphorus was abnormal in Pens I and II. In Pen I the ewes received over five times as much calcium as phosphorus, and in Pen II between three and four times as much phosphorus as calcium.

The experimental ewes were exercised twice daily by turning them into a large brick-floored pen where they were kept moving by systematic disturbance. They were weighed in December, March, April and May, and at the same time small samples of blood were withdrawn from the jugular vein for determination of hæmoglobin, serum calcium and inorganic phosphorus. Although the change from pasture to dry food was made gradually, great difficulty was experienced in getting the ewes to consume their trough ration completely, especially as the concentrates were of a powdery nature. Blackface ewes are seldom hand-fed or housed in any way, except when prevented from access to their natural food by snow. The hay was ravenously eaten, but the maize-starch and blood-meal mixture only reluctantly accepted. Practically all the animals suffered a set-back in weight, but after chopping up part of the hay ration finely, mixing well

TABLE III.—RECORDS OF BODY WEIGHTS AND LAMBING DETAILS.

Experiment No.	Sheep No.	Dec. 12 lb.	Mar. 7 lb.	Apr. 3 lb.	May 18 lb.	Date of lambing	Weight of lamb lb.	Lambing comments
I.—Pen I.	8	75	79	86	75	14.4.33	7	Normal lamb, ewe died 22.5.33
Low phosphorus	40	76	84	94	79	6.5.33	6	Normal lamb, ewe very weak
	15	84	90	104	79	20.4.33	—	Aborted 20.4.33
	44	84	100	104	96	9.5.33	5	Normal lamb
	32	80	88	92	—	21.4.33	7	Normal lamb, ewe died 1.5.33
II.—Pen II.	16	95	109	119	83	21.4.33	5½, 7½	Normal twin lambs
	23	92	100	105	—	24.4.33	7½	Normal lamb, ewe died 19.5.33
Low calcium	6	65	87	100	66	13.4.33	10½	Normal strong lamb
	34	87	92	83	76	22.5.33	6	Normal lamb, ewe very weak
	2	83	81	93	—	10.4.33	—	Aborted 10.4.33, ewe died 10.5.33
III.—Pen III.	3	83	103	113	76	13.4.33	8, 10	Normal twin lambs
	35	100	116	124	84	20.4.33	7	Normal lamb
Low phosphorus, low calcium	37	86	102	101	91	2.5.33	6	Normal lamb
	17	88	99	101	73	19.4.33	6, 6	Normal twin lambs
	42	72	79	75	57	21.4.33	6	Normal lamb
IV.—Pen IV.	11	72	77	78	—	—	This pen was turned out to pasture on April 15th, owing to the extremely weak condition of the ewes. They recovered quickly on grass and all gave birth to normal lambs	
	25	70	76	80	—	—		
Low phosphorus, low calcium. Cod-liver oil added	18	70	—	72	—	—		
	4	80	81	112	—	—		
	36	82	89	99	—	—		
V.—Pen V.	24	75	91	96	70	22.4.33	11	Normal strong lamb
	19	88	110	105	—	17.4.33	7, 7½	Normal twin lambs, ewe died 24.4.33
Adequate phosphorus and calcium	12	79	90	96	70	14.4.33	7	Normal lamb
	45	91	96	97	102	20.5.33	6	Normal lamb
	38	81	84	—	—	23.3.33	—	Ewe died before lambing, 23.3.33

with the concentrates and minerals and increasing the allowance of salt, improvement in food consumption followed, and during February their condition improved materially. Table III records the body weights over the period of experiment and provides lambing details.

It will be noted that the March weights exceed the December weights in all cases except one (Sheep 2, Pen II), so that the initial set-back accompanying the change from pasture to experimental conditions was not such as to invalidate the experiment. Thereafter twenty-one out of the twenty-five continued to gain in weight until they lambed. Four ewes (Nos. 34, 37, 42 and 19) showed an April drop on the March figures. Individual records of food consumption were not made, so that it is not certain how far this fall in weight was due to failure of these ewes to consume their full share of the rations. It should be added that since the mineral supplements were mixed with the concentrates, failure to consume the full allowance would also result in reduced daily mineral intake.

Results and Discussion.

Lambing.—There were two cases of abortion of a non-contagious character: Ewe No. 15 (Pen I), on a low phosphorus diet, and ewe No. 2 (Pen II), on a low calcium diet. One ewe (No. 38, Pen V), died before lambing. The remaining twenty-two animals lambed normally. The majority of the lambs throve badly owing to the paucity of milk secreted by the mothers, but the significant feature of the lambing is that none of them showed any clinical signs of deformity or skeletal abnormality. The bones appeared quite normal and no signs of "cripples" could be detected by the most careful observation. Even on the rations deficient in calcium, or in phosphorus, or in both, normal lambs were born even when the ewes themselves showed signs of general malnutrition. That many of the ewes were undernourished was obvious from their general appearance and difficulty in recovering after lambing. The data provided by blood analyses frequently point in the same direction and show distinct anæmia. The blood data also clearly indicate incipient aphosphorosis on the phosphorus-deficient diets, while the fall in blood calcium on the calcium-deficient rations is quite distinct, although much nearer the normal and not necessarily very significant.

It is, therefore, reasonable to conclude that "cripples" in lambs is not due to mineral deficiency in the rations of the ewes. At least this experiment provides no support for a mineral-deficiency theory of causation, and, taken in conjunction with the field experiment already recorded,[1] provides strong evidence against it.

Blood Analyses.—The results of the blood analyses are given in Table IV. Hæmoglobin is expressed in "sheep scale units," *i.e.*, on a scale in which the average amount of hæmoglobin in normal sheeps' blood is taken as 100, as explained in a previous article.[1] The actual determinations were made with a Klett colorimeter and a Newcomer disc, read as "grammes hæmoglobin per 100 c.c. of blood," and then converted to easily comparable percentage figures by taking the normal value for sheep as 11.45 gm. per 100 c.c. Thus the average of the

TABLE IV.—DATA OF SERUM CA, SERUM INORGANIC P AND HÆMOGLOBIN IN THE BLOOD OF EXPERIMENTAL SHEEP.

Pen No.	Sheep No.	Serum Ca. mg. per 100 c.c.				Serum Inorganic P. mg. per 100 c.c.				Hæmoglobin Sheep Scale Units 11.45 gm. Hb. per 100 c.c. = 100			
		Dec. 12	Mar. 7	Apr. 3	May 18	Dec. 12	Mar. 7	Apr. 3	May 18	Dec. 12	Mar. 7	Apr. 3	May 18
Pen I.	8	10.90	10.40	9.40	9.60	5.63	2.34	1.90	2.01	103	82	61	67
	40	11.50	11.90	11.10	9.70	5.07	2.65	2.11	2.42	96	84	94	66
Low P.	15	11.50	10.30	9.40	9.50	5.66	2.25	2.00	3.19	94	76	69	81
	44	11.50	11.60	10.30	10.00	5.15	2.70	2.00	2.70	90	84	86	89
	32	11.30	10.70	11.40	died	5.30	2.72	2.17	died	95	93	83	died
Av.		11.34	11.00	10.32	9.70	5.36	2.53	2.04	2.58	96	84	79	76
Pen II.	16	10.60	8.50	7.80	8.90	6.03	3.97	6.37	5.40	83	86	84	88
	23	10.80	7.90	8.00	8.20	5.09	4.50	6.25	4.12	100	99	83	65
Low Ca.	6	11.90	8.30	8.30	8.40	5.63	4.82	7.50	4.60	98	100	77	79
	34	11.80	7.40	6.00	6.90	5.40	5.19	5.82	4.22	100	92	83	69
	2	11.70	6.00	6.20	died	5.31	3.86	4.82	died	83	80	62	died
Av.		11.36	7.60	7.26	8.10	5.49	4.47	6.15	4.59	93	92	78	75
Pen III.	3	11.10	9.80	9.10	10.10	5.84	—	2.07	3.14	104	96	65	52
	35	11.70	11.00	9.50	10.00	4.79	3.46	2.00	3.10	93	91	79	79
Low Ca.	37	11.25	10.30	9.00	9.30	—	4.36	2.21	2.62	82	70	94	104
Low P.	17	11.60	10.50	9.20	9.00	4.82	2.91	2.30	3.25	91	90	71	58
	42	10.70	7.90	9.60	8.70	4.55	3.59	2.10	3.28	84	82	66	54
Av.		11.27	9.90	9.28	9.42	5.00	3.58	2.14	3.08	91	88	75	69
Pen IV.	11	10.65	10.10	9.20	—	4.69	2.70	2.21	—	86	88	76	—
As in III	25	10.75	9.60	8.90	—	5.31	2.61	2.01	—	86	87	70	—
plus	18	10.70	—	9.30	—	5.71	—	2.08	—	78	—	70	—
C.L.O.	4	11.05	10.60	10.10	—	3.81	2.81	2.01	—	80	77	85	—
	36	11.70	10.20	10.40	—	5.63	3.01	2.33	—	95	92	86	—
Av.		10.97	10.13	9.58	—	5.03	2.78	2.13	—	85	86	78	—
Pen V.	24	10.20	9.20	8.00	8.00	5.10	5.04	4.57	6.75	100	95	83	100
	19	10.40	9.70	9.00	died	4.95	4.14	4.50	died	86	86	84	died
Adequate	12	11.45	8.50	8.70	8.10	5.04	5.13	4.56	5.53	88	87	71	100
Ca. & P.	45	11.10	9.90	10.60	9.90	4.85	4.56	6.31	5.87	87	85	69	79
	38	11.50	8.70	died	—	4.98	4.88	died	—	86	82	died	—
Av.		10.93	9.20	9.08	8.67	4.98	4.75	4.99	6.05	89	87	77	93

five sheep in Pen I was 10.96 gm. hæmoglobin per 100 c.c. blood, and is therefore shown in Table IV as 96, or four per cent., below the average previously determined for healthy sheep.[1] Calcium and inorganic phosphorus are expressed in the conventional way as " milligrams per 100 c.c. of serum."

(i) *Hæmoglobin.*—It will be noted that even before the experiment commenced, most of the sheep showed hæmoglobin values somewhat below the average. The range from highest (No. 3) to lowest (No. 18) is 104 to 78, but since the normal range of sheeps' blood is fairly wide no great significance is attached to this, and none of the sheep can be regarded as seriously anæmic. What is significant, however, is the fact that twenty-two out of the twenty-five ewes show a fall in hæmoglobin value from December to April, *i.e.*, as the lambing period approaches. This is most marked for some of the ewes on the mineral deficient rations (*e.g.*, No. 8, which fell from 103 to 61), but is also shown by the sheep in Pen V, which received a mineral supplement ensuring adequate calcium and phosphorus (*e.g.*, No. 24, which fell from 100 to 83). Taking the average values for the five sheep in each pen, there is in every case an appreciable drop in the hæmoglobin figure, of sufficient magnitude to indicate a distinct " nutritional anæmia," as pregnancy advances on the experimental diets. In how far this is due to pregnancy as such, and how far it is due to the imperfect general character of the basal ration, it is difficult to say. Both factors are presumably involved, since some of the sheep show recovery on the same diet a few weeks after lambing (*e.g.*, Nos. 24, 12, 37, 16 and 44), while others become progressively more anæmic (*e.g.*, Nos. 3, 17, 42, 34, 23 and 40). (It is interesting to note at this point that a similar drop in the hæmoglobin figures was recorded in the sheep used in the field experiment described in a previous article.[1] These sheep were existing under natural conditions and grazing on natural pasture, supplemented in some cases by a special cake.) Since the differentiation between pens is not sharp, however, this general tendency to anæmia is not the most striking feature of Table IV. Of greater interest are the figures for blood phosphorus.

(ii) *Inorganic Phosphorus.*—The average figure for Pen I before experimental feeding commenced is shown as 5.36 mg. per cent. inorganic phosphorus. By March 7th, after less than three months of feeding on the phosphorus deficient ration, the average figure had fallen to 2.53 mg. per cent., a drop of 53 per cent. The April figure is even lower, 2.04 mg. per cent., corresponding to a fall of 62 per cent. from the original value. After lambing the average value returns to the March figure, *i.e.*, remains less than half the December value.

The data for Pen III, deficient in both calcium and phosphorus, and for Pen IV., also deficient in these elements, but supplied with cod-liver oil, are similar. In both cases the April value is only about 42 per cent. of the December value.

In marked contrast to this is Pen II, on low calcium but high phosphorus, and Pen V, adequate in both elements. In both cases the April value is as high as the original December value, and although some fluctuations are shown, they may be regarded as within the normal range. Taking the sheep individually,

it is plain that on the phosphorus-low rations every ewe without exception shows a marked hypophosphatæmia. It is, therefore, safe to conclude that these ewes were suffering from "aphosphorosis," and that if they had continued on the phosphorus-deficient ration they would ultimately have developed clinical symptoms of skeletal disease.[5]

(iii) *Serum Calcium.*—The figures for calcium are also of interest, although more difficult to interpret than those for inorganic phosphorus. In every pen the average value of all five ewes falls somewhat from December to April. The decrease is most marked on the calcium-low rations, but since it is also shown by Pens I and V, in which dietary calcium was adequate, it is not easy to account for. Setting the average December value of each pen as 100, the April figures work out at 91, 64, 82, 87 and 83 for Pens I, II, III, IV and V respectively. Since the fall for Pen V with both calcium and phosphorus-high is the same as for Pen III with both elements low, the much more marked fall for Pen II on low calcium and high phosphorus cannot be attributed to the low calcium alone. In this pen, however, phosphorus was high, the ratio CaO : P_2O_5 being 1 : 3.26. It is tempting to conclude that the effect of the adverse ratio and relative excess of phosphoric acid over calcium oxide imposed a drain upon the calcium reserves of the body and depressed the level of blood calcium; but without further evidence such an hypothesis is perhaps unwarranted.

The general trend of the figures may be indicated by pointing out: (*a*) that serum calcium showed a fall as pregnancy advanced upon rations which were inadequate in the general sense as judged by the appearance of the ewes and the tendency to anæmia; (*b*) that this fall was most marked on the ration with low calcium and high phosphorus, and least marked on the ration of high calcium and low phosphorus; and (*c*) that the average fall on the ration in which both elements were low (Pen III) only amounted to 18 per cent., a small figure compared with the large drop of 57 to 62 per cent. in inorganic phosphorus on those rations deficient in phosphorus. It would seem that, in general, low phosphorus in the ration of ruminants is rapidly reflected by a fall in inorganic phosphorus in the serum, but that low calcium in the ration does not influence the blood level of calcium to the same extent, probably because the level of blood calcium is under the control of a definite physiological mechanism in which the parathyroid glands play a prominent part, and therefore tends to remain more constant in spite of dietary deficiencies. These observations are in line with the South African work of Theiler, Green, Du Toit, Malan, Rossouw and others, although not generally accepted by observers elsewhere.

The normal range of blood calcium may be taken as about 8.5 mg. per cent.–12 mg. per cent. Figures beyond this range are sometimes encountered, but 8 mg. per cent., would generally be regarded as definitely low, although not necessarily beyond the limit for healthy functioning of the mineral metabolism of the animal. In Pen II all the values approached 8 mg. per cent. or fell below it, and two of the sheep showed extraordinary low values. In April, No. 2 showed

6.3 mg. per cent., and No. 34 showed 6 mg. per cent., representing a fall to about half the original December values. These are regarded as definitely beyond the normal physiological range, but no definite view is put forward to explain them. They may be related to the diet, but it is equally possible that they are due to some disturbance of the physiological mechanism for controlling the level of blood calcium. Attention is drawn to them mainly because they are unusually low values for sheep.

(iv) *Cod-liver Oil.*—The influence of cod-liver oil upon the level of blood calcium and phosphorus in Pen IV is not pronounced. Although the drop in blood calcium is somewhat less than in Pen III the difference is not significant. The addition of cod-liver oil, rich in vitamins A and D, did not prevent the inorganic phosphorus from falling from 5.03 mg. per cent. in December to 2.13 mg. per cent. in April, *i.e.*, to about 42 per cent. of the original value. The fall is therefore definitely due to phosphorus deficiency of the ration, and not due to shortage of vitamin D, the factor to which the fall in blood phosphorus is usually attributed in the case of rickets in children.

In concluding this survey of Table IV, and of the experiment in general, it is regretted that for financial reasons the trials could not be pushed to their logical conclusion, and all the animals maintained on the mineral-low rations until definite clinical symptoms of disease appeared. But the general falling off in condition of all the animals suggested that the basal ration would have to be modified even in the case of the control Pen V, and it was decided to abandon the experiment rather than lose the remaining sheep. Furthermore, the experiments of Theiler, Green and Du Toit[6] on cattle, indicate that clinical symptoms do not develop in aphosphorosis of ruminants for a very long time (fourteen to twenty months) after deficient feeding is commenced, while the subsequent experiments of Du Toit, Malan and Rossouw[7] on sheep indicate that the level of inorganic phosphorus in the blood may show diminution even after a week of phosphorus-deficient feeding. Our own sheep, although they showed such a pronounced drop in inorganic phosphorus of the blood within two months of commencing the experimental rations, might therefore not have shown definite clinical symptoms of disease for another year, a period longer than we could afford to carry through the experiment.

Nevertheless, the experiments may be taken as achieving their main object by supporting the conclusions arrived at in the field experiment recorded in the first paper,[1] and providing strong presumptive evidence that the disease of "cripples" in lambs is not related to mineral deficiency in the diet of the maternal ewes.

Summary.

(1) Pregnant ewes were fed in five lots of five for periods up to five months on rations of varying mineral content, compounded by adding mineral salts to a basal ration of maize starch, blood meal and poor hay.

(2) The ewes on phosphorus-deficient diets showed a marked fall in the inorganic phosphorus of the blood; in less than two months this had fallen to about half the original figure.

(3) A distinct fall in blood calcium was observed on all the diets, most marked on a ration with low calcium and high phosphorus, and least marked on a ration with high calcium and low phosphorus. The average fall of blood calcium on a ration low in both elements was, however, only 17 per cent. as compared with 62 per cent. for inorganic phosphorus on a phosphorus-low diet.

(4) All the ewes suffered to some extent from general malnutrition, as the result of change from pasture to the imperfect experimental diets and adverse experimental conditions of confinement, and a distinct anæmia was observed as pregnancy advanced. In some cases hæmoglobin returned to normal after lambing, in other cases not.

(5) Despite malnutrition and deficiency of phosphorus, or of calcium, or of both, the lambs were born normal. The majority throve badly as a result of shortage of milk in the ewes, but there were no clinical signs of "cripples," or of any skeletal abnormality. The condition known as "cripples" in lambs, therefore, is not due to mineral insufficiency of the rations of pregnant ewes, a conclusion supported by a field experiment on a "cripples" farm in which a mineral supplement failed to reduce the natural incidence of the disease.

Acknowledgments.

Grateful thanks are due to Mr. W. Lyle Stewart, M.R.C.V.S. (in whose department the work was executed), for much assistance and advice during the experiments; to Dr. Andrews, of the Ministry's Laboratory at Weybridge, for kindly co-operation and encouragement; and in particular to Dr. Green, also of the Weybridge Laboratory, for continual advice and criticism both before and during the investigation, and in the writing of this paper.

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STUDIES ON THE SO-CALLED ELECTRICAL STUNNING OF ANIMALS*

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Introduction.

FOR some years past electrical current has been employed in abattoirs in order to render animals unconscious before killing them. In many cases the alternating current is used. In this Journal papers have been published on this subject. The apparatus used has been called the "electrolethaler."

One is inclined to suppose that before using the electric current for this purpose physiological facts and experimental data have been supplied showing that in all probability the animals treated in this way are actually brought into a state of unconsciousness or analgesia. The literature on this subject, however, does not confirm this supposition. Investigations of this subject are scarce, and in most papers in which the so-called stunning of slaughter animals by an alternating current is treated, the experiments of Leduc[6 7] with interrupted constant currents are cited, even although the action of the constant current differs fundamentally from the effect of the alternating current. Hitherto the condition into which Leduc brought his animals differed essentially from what is generally accepted as being the state of narcosis. We shall give a brief explanation of this.

Leduc[6] found that a constant current, preferably of 5–20 volts, rhythmically interrupted 85–90 times in a second in such a way that a fixed relation was maintained between the duration of the current and the interval, was able to bring rabbits into a comatose state like narcosis, when the strength of the current was a few milliamperes and the animals had been subjected to it from three to five minutes. He afterwards experimented on himself, and describes the results as follows†: "At first the sense of speech was inhibited. Afterwards the motor centres are completely inhibited. It was impossible to react to stimulations, even to the most painful ones. . . . We were conscious of the impotence to move; we felt the contacts, etc. . . . Respiration was impossible; it seems that when death supervenes, it must be imputed to this fact." Also in other experiments on himself the motoric functions proved to be paralysed, while the high cerebral functions were not attacked at all, or merely in such a degree that perception and asthesia persisted.

It is not clear why this syndrome was called electronarcosis by Leduc; this name suggesting the state of narcosis evoked electrically. When we try to summarise the symptoms of the chemical narcosis the following picture may be given: Narcosis may be described as being a state resembling sleep, in which activity of many cells, especially those of the central nervous system, is abolished

* Received March 22nd, 1934.—ED. † Translated from French.

and reflexes fail completely or in part ; the number of reflexes that are wanting depends on the depth of the narcosis. If narcosis is not deep, only the highest reflexes are absent.

Narcosis is, furthermore, characterised by the selectivity of the narcoticum. The part of the reflex path that lies in the central system, *i.e.*, in the spina, is affected first. But the connections between the spinal centres and the higher parts are already previously narcotised. It is by this connection that sensations can accompany reflexes in the higher animals. Narcosis owes its merit in surgery just to this selectivity, even if the surgeon proceeds giving narcosis in order to avoid hindering of his work by the reflex. On the other hand, it is necessary that the automatic centres indispensable for life are spared by the narcotic. The value of the narcotic is essentially determined by the margin left free between the block of the spinal ganglion cells mentioned and the medulla oblongata, which contains the automatic centres without which life is impossible.

Comparing the characteristics of narcosis with the symptoms described by Leduc, fundamental differences may be noted. Leduc's motoric centres were inhibited completely, as well as his respiration, whereas consciousness and observation persisted. He was even able to discern that the stimulus was painful and to perform mental processes. Reactions on painful excitations were, however, impossible. From this it must be concluded that the central nervous system was affected in quite another way than in narcosis. For in Leduc's experiments (*a*) consciousness and sensation were present, whereas motoric centres were already involved, and (*b*) the medullar respiratory centre was affected, however weak he had chosen the current. It is easy to understand that Leduc's attempt to attain a physical narcosis for men in the way described had no success during the forty years that have elapsed since his experiments.

Leduc's currents found application in apparatus constructed for stunning animals. This way of stunning was soon replaced by the alternating current for technical reasons. The procedure was simplified by this. This current was available in most towns, and the motor and the moving contacts of Leduc's scheme could be now omitted. The question, however, as to whether the alternating current was a better warrant that the animals were really stunned was not regarded in this change. On the other hand, it is surprising that Leduc's experience,[4] that the alternating current is unsuitable for the stunning of animals, has neither found application nor been brought under discussion.

Method.—The apparatus used contains a transformer which transformed the 220 volts of the town into a current of 50, 60 or 70 volts as wanted. As to its construction and the electrodes used, it corresponds with the co-called electrolethaler known to the readers of this journal. According to the instructions of the constructor of this apparatus, the contact with the animal was made by placing the electrodes, which are combined to a forceps, on either side of the skull under the ears, after having saturated them with a 20 per cent. salt solution. These electrodes had a surface of 35 cm. for the pig and the calf, while for the

smaller animals smaller electrodes were used, with about 7 cm. surface. The current passed the skull 10–15 seconds.

The cat, the dog, the rabbit (grown-up animals), the pig (weighing 20–45 kg.), young goats (three months old) and the calf (about one week) were used in the experiments. Currents of 60 or 70 volts were commonly chosen; in small animals currents of 50 volts were sometimes used. This difference in current did not notably influence the results. In some experiments closure of the circuit was maintained longer than fifteen or shorter than ten seconds; a special note will be made of this. A micro-amperemeter and a variable resistance placed in the secondary circuit enabled us to read and to vary the strength of the current. This precaution is useful for two reasons: (*a*) the difference in resistance between different animals even of the same species is such that the strength of the current, if not regulated, may vary 50 per cent. and more; (*b*) the resistance of the animal, which is to a great degree resistance of the skin, may vary during the time that the current passes. In all animals, with the exception of pigs, the hairs on the spot where the electrodes will be placed are previously cut, and the skin is moistened with the salt solution, care being taken that the surrounding skin is left dry. The animals are placed on an adjustable table, the leaf containing four holes for the legs. In this way the animal is free to move, and the movements may be easily observed.

Results.—When the circuit is closed and the current of about 300 milliamperes passes, the animal exhibits symptoms which will not be circumstantially described here, but which will be summed up in the following groups:

(1) Tonic and clonic contractions of all skeletal muscles. The tonic spasms precede the clonic contractions, which are often followed by convulsions. These tonic contractions are not physiological ones. The normal reciprocal innervation between extensors and flexors being disturbed, both groups contract simultaneously, which is never the case under physiological circumstances. So the final position of a leg is determined by the forces of the different groups of muscles counteracting each other. The contractions are the effect of the stimulation of the nervous system; in a curarised animal the spasms fail. By using spinal cats, *i.e.*, cats in which the spinal part is separated from the higher parts of the central nervous system, it could be demonstrated that the spinal centres are also stimulated. According to the smaller density of the current, this happens in a lighter degree than in the centres lying in the skull and its neighbourhood.

The respiratory movements are arrested during a time, this varying in different experiments from twenty-five seconds to three-quarters of a minute. Particularly in the cat, the tail is stretched upwards perpendicularly, while the hairs thereof bristle very strongly, so that it resembles a beautiful duster as used for the chimney of a lamp. The hairs of the neck and of the back also bristle through excitation of the sympathetic system. By these sympathetic symptoms, the extended legs and the cyphosis of the body, the animal strikingly resembles an angry cat

ready to pounce upon its enemy. These symptoms are prolonged after the current is broken ; the hair erection may even continue as long as six minutes.

(2) Other sympathetic symptoms are likewise observed in the smooth muscles of the bulbus oculi, the iris and the eyelids. Parasympathetic symptoms are observed too. As to the eye, an equilibrium is obtained between the former or the latter which changes in the different species of animals and according to the moment chosen. In the end the sympathetic effects predominate in all animals, but at a different moment and in a different degree. The mydriasis may be observed during three-quarters of a minute. The cat and the dog exhibit ptyalism.

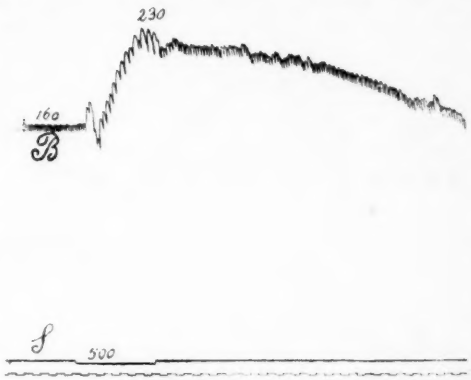

FIG. 1.—Arterial blood-pressure in the cat. B = arterial blood-pressure ; S = signal for passing the current ; The numbers on the blood-pressure tracing indicate the pressure in mm. Hg. ; the number on the S line the strength of the current in ma. This also holds for the next figures.

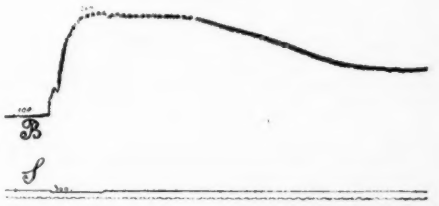

FIG. 2.—Arterial blood-pressure in the cat after both vagi have been cut high on the neck. B = arterial blood-pressure ; S = signal for passing the current.

(3) Excitation of medullar centres from which the respiratory centre, the vaso-motor centre and the vagus centre have been studied. Nystagmus horizontalis, either to the right or to the left side, will last in the cat for some minutes. All the symptoms mentioned persist some time after the current is broken. As to the spasms of the skeletal muscles, one could suppose that they stop the moment the current is stopped, as the animal relaxes then to a certain degree. This is not the case, however, in either animal, strong action currents of the muscles being detected by the string galvanometer during half a minute and longer, and after this period a period of convulsions may occur, taking half a minute or more. Sometimes this period coincides with the period of tonic spasms, or may follow it immediately.

(4) Rise of temperature from 0.3° to 0.7° C.

In order to study the effect of the current on the medullar centres, the blood pressure of the art. carotis and the respiration was recorded. In Fig. 1 a record is given, showing a rise of blood pressure from 160 to 230 mm. Hg. At the moment

the current is interrupted blood pressure has reached its maximum, and afterwards it returns slowly to its normal level. The rising part of the record exhibits vagal pulsus in consequence of stimulation of the vagus. These disappear after both vagi have been sectioned in the neck. Consequently the rise of pressure increases by this procedure, as Fig. 2 shows, where blood pressure rises more than 100 per cent. (from 108 to 264 mm. Hg.). Fig. 2 shows the effect on the vasomotor centre without the compensating influence of the cardiac vagal centre.

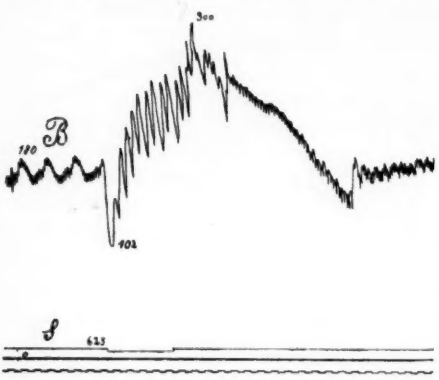

FIG. 3.—Arterial blood-pressure in the pig. B = arterial blood-pressure; S = signal for passing the current.

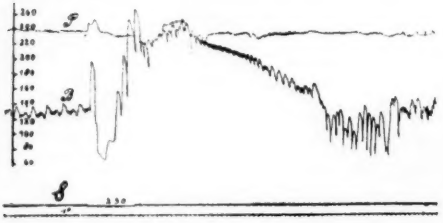

FIG. 4.—Arterial blood-pressure in the goat. B = arterial blood-pressure. The numbers on the left indicate mm. Hg. The rest as in Fig. 3.

In Fig. 1 the rise of pressure is preceded by a slight fall; it is caused by the preponderance of the vagal effect at this moment, as is proved by Fig. 2. Here the effect has disappeared. In the pig and in the goat this initial fall is still more pronounced, as is demonstrated in Figs. 3 and 4.

The respiratory movements were recorded by connecting the recording tambour with the trachea, an air reservoir being placed between the animal and the tambour from which fresh air could be taken. In this way the arrest of the respiratory movements was recorded, as well as the phase in which this took place. The strength of the current determines the position of the arrested thorax. In the cat, currents of 40 ma. and higher arrest the thorax in inspiration, whereas weaker currents will cause arrest in the expiratory position or near about this. The difference between these two effects is very sharply pronounced. When a current of 40 ma. is used it can often be observed that the expiratory position in which the thorax is first arrested gradually merges into inspiration position, or very nearly to this, during the time the current passes. In Fig. 5 an example is given. In still another regard currents of 40 ma. constitute a border-line. When weaker currents are used the effect on the respiratory movements is limited to the time the current passes; stronger current may arrest them during a longer time—half a minute or longer.

The effect on the respiration depending on the strength of the current described here agrees completely with the results obtained by stimulating afferent respiratory vagus fibres. Therefore we must conclude that the respiratory movements are arrested in a specific way, this being subject to the strength of the stimulation of the respiratory centre. The respiratory centre is very sensitive to the current furnished by the apparatus. Currents of 2–3 ma. already affect its activity, the effects depending on the sensitiveness of the preparation; the respiratory movements are often increased in depth and in frequence. Currents stronger than 10 ma. have a more constant effect and act in the way described above.

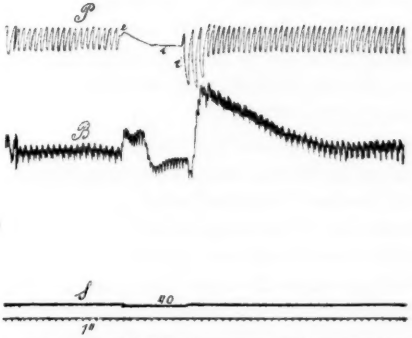

FIG. 5.—Arterial blood-pressure and respiratory movements in the cat. Strength of the current, 40 ma. P=pneumogram; e=expiration; i=inspiration; B=arterial blood-pressure; S=signal for passing the current.

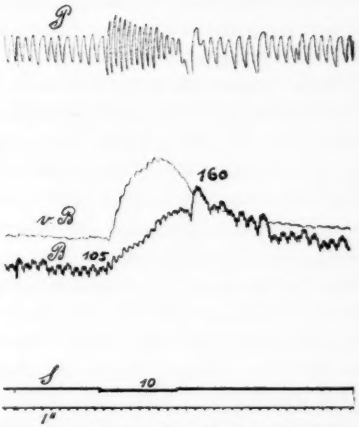

FIG. 6.—Arterial and venous blood-pressure in the cat. Strength of the current, 10 ma. P=pneumogram; v B=venous blood-pressure; B=arterial blood-pressure; S=signal for passing the current.

From 10 ma. upwards the vasomotor effect is obvious, the efferent vagus effect being most often absent when a current of 10 ma. is used. A current of 20–30 ma. excites all the three centres mentioned from the beginning. In Fig. 6 a record is given, showing excitation of the three centres by a current of 10 ma.

Comparison Between the Effect of the Alternating Current Narcosis.

When comparing the effects of the electric current used with those of narcosis, fundamental differences are met with. In narcosis the activity of the motor centres is not increased, but diminished; in deep narcosis it may be totally destroyed. In narcosis no spasms of clonic convulsions are found, but, on the contrary, relaxation of skeletal muscles is met with. Especially the effect on the oblongata is quite another one. Whereas it is characteristic for narcosis that the medullar centres are spared, the latter are attacked very violently by the alternating current.

In order to get a better insight into the effect of the current on the central nervous system, the following experiments were performed: (1) Narcotised animals were subjected to the current; (2) thalamus cats were subjected to it; (3) Some phenomena, generally used to judge the stage of stunning, were studied.

(1) (*a*) If animals are narcotised with a volatile narcotic (ether for the cat, ether + chloroform for the dog, the rabbit and the goat) before the current passes, the clonic spasms decrease or disappear according to the depth of narcosis. This fact is in agreement with the view that the electrical current acts as an excitation on the central nervous system. It is difficult to abolish the tonic spasms by narcosis. (*b*) The duration of narcosis is not lengthened when the animal is subjected to the full current during narcosis. In some experiments the narcosis was even abbreviated by the current. (*c*) Incomplete narcosis to such a degree that the cornea reflex was still elicitable is not completed by the electric current if the latter is not so strong that it abolishes the cornea-reflex itself. (*d*) It is not to be wondered at that the medullar centres are as strongly affected in the narcotised animals as in the normal ones; they are spared by the narcoticum, and therefore they remain unprotected for the electric current.

(2) If the hemispheres are extirpated in cats before the current passes the skull, animals exhibit the same symptoms as the normal ones, with the exception that the clonic spasms are either absent or scarce. It is needless to note that the pupil phenomena will fail, the latter remaining in maximal mydriasis in consequence of the operation. As is the case in the normal cat, the cornea reflex is positive here again in twenty to thirty seconds after the current is broken. The tonic spasms are not less than in the normal animal. This result proves that the suggestion already given by the animals in narcosis, *viz.*, that the hemispheres are not indispensable for the tonic muscle spasms, is correct. Great importance has been attached to these spasms by Müller. This author has written a lot of papers and theories on the subject which need not all be discussed: so far they do not start from physiological facts or generally accepted scientific reflections. As to the question of the spasms, Müller[9] gives the theory that especially the " tonic spastic effect on the muscles and the vessels " prove that the normal functions of the hemispheres are abolished.

That the state of the blood vessels could enable conclusions to be drawn as to the activity of the brain was *a priori* unlikely, as the vasomotor centre in the oblongata rules the condition of the blood vessels. Neither could it be expected that the spasms of the skeletal muscles have this meaning. It was proved by the experiments on thalamus cats that such spasms cannot inform us as to the state in which the hemispheres are brought by the electric current; the tonic spasms persist even although the preparation does not have hemispheres at all.

(3) The electric current does not act as a protecting influence on the centres. (*a*) A reflectory rise of blood pressure can be elicited by stimulating an afferent nerve, *i.e.*, the central stump of the cut n. peroneus. Narcosis extinguishes this reflex, so that even a stimulus stronger than effective before is not able to evoke

it now. Such a stimulus is, however, efficient shortly after, sometimes even while the current is passing through the animal, notwithstanding that the blood pressure is already highly increased by the current itself. This effect was proved to be the result of the action on the bulbar vasomotor centre, which is neither protected nor paralysed by the electric current. Nor could any protecting action on the motoric centre be observed, as it is found in chemical narcosis. (*b*) Stimulation of an afferent nerve also evokes a reflectory mydriasis, which disappears when the animal is brought under narcosis. For a moderately strong stimulus the path for the pupil dilation is blocked during narcosis, and when narcosis is stopped after it has been maintained for some minutes with a fixed dosis of the narcotic, the reflex will return after a time varying from four to ten minutes in the cat. This time was found to be much shorter when narcosis was replaced by the electric current. Under these conditions ten to seventy seconds will do.

This observation in itself is not necessarily surprising. It would tally with the suggestion that the change in the nervous system caused by the electric current will vanish sooner than the change after narcosis, but agrees with it as to its character. This view is, however, untenable, the time given here not being entirely controlled by the condition of the nervous system. It is influenced, too, by the fact that the pupil still being fixed in a position of mydriasis by the current, is prevented from reacting sooner. Also, if these centres themselves and the path to and from the centre were fully free, it would be impossible to observe any effect, the effector organ, the pupil, being unable to demonstrate that fact. Consequently the time of from ten to seventy seconds is really too high; it only indicates the moment to which the pupil is again free to react. In Table I the close relationship between the time needed by the pupil to be free to react again, and the recovery of the pupil reflex, is demonstrated.

TABLE I.—REFLECTORY MYDRIASIS IN THE CAT.

The electric current passed the skull during	Strength of the current	Time wanted by the reflectory mydriasis to be elicitable after the current was broken	Electric mydriasis begins to vanish after :
10 seconds	300 ma.	50 seconds	45 seconds
10 ,,	,, ,,	15 ,,	15 ,,
10 ,,	,, ,,	10 ,,	10 ,,
5 ,,	,, ,,	35 ,,	30 ,,
5 ,,	,, ,,	45 ,,	40 ,,
5 ,,	,, ,,	55 ,,	35 ,,
5 ,,	,, ,,	25 ,,	25 ,,
5 ,,	,, ,,	20 ,,	20 ,,
5 ,,	,, ,,	40 ,,	35 ,,
5 ,,	,, ,,	35 ,,	30 ,,

The action of the alternating current on the central nervous system being characterised by its strong excitatory effect, the question must be put as to whether this effect is followed by a state of fatigue or exhaustion of the centres or not. Fatigue or exhaustion in consequence of augmented activity is not an exception

in physiological phenomena. None of the centres before mentioned showed any sign of fatigue after they had been submitted to the electric current in the manner described. The arrest of the respiratory movements after the current was broken is not a sign of exhaustion of this centre ; on the contrary, it is the consequence of its increased activity, as it was also during the time the current was led through the animal. If the circuit is closed again shortly after it was opened, the animal exhibits all the symptoms already mentioned again, the muscle spasms as well as the others. This may be repeated several times. In Fig. 7 an example for blood pressure is given as observed in a cat, which was subjected during half a minute to a current of 500 ma.

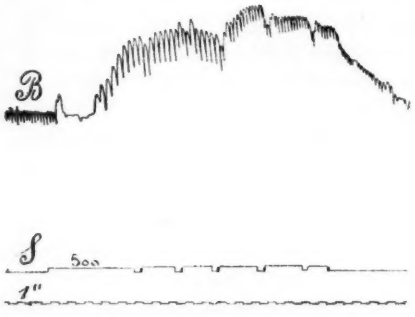

FIG. 7.—Blood pressure in the cat. A current of 500 ma. is given during about half a minute. Blood pressure falls on every interruption of the current and rises again as soon as the circuit is closed. B = blood pressure ; S = signal for passing the current.

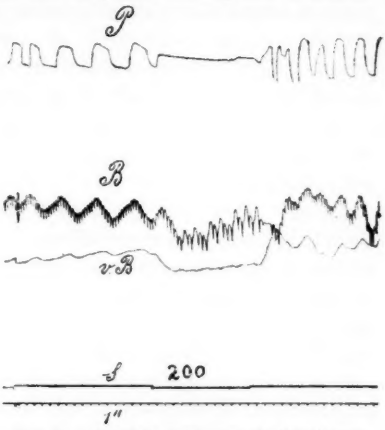

FIG. 8.—Arterial and venous blood-pressure in the cat. A current of 200 ma. is given for the twelfth time. The vasomotor effect failing, arterial blood-pressure falls in consequence of the persisting vagal effect on the heart. The respiratory centre is also still intact. Venous blood-pressure falls as muscle spasms fail or are scanty. P = pneumogram ; B = arterial blood-pressure ; v B = venous blood-pressure.

When the animal has been submitted to the experiment ten times or more, with intervals lasting not longer than a few minutes, the moment will come that the centres begin to be paralysed. The vasomotor centre is the first of the medullar centres to be exhausted, the respiratory centre follows; the vagal centre is of the three the most resistant (Fig. 8). It should be emphasised, however, that such symptoms of fatigue only present themselves after the experiment has been repeated many times. Should the current be applied during the time prescribed, or even should this period be surpassed by 100 per cent., there is no trace of fatigue or of exhaustion, neither of the medullar centres mentioned, nor of the motoric ones.

As others already have observed, some animals may exhibit symptoms of having unpleasant recollections after being treated with the electric current.

Kok and Van Harreveld[5] used an alternating current; Von Neergaard[10] and Zimmermann[14] an interrupted constant current. Battelli[1] described agitation in the dog that had been submitted to an alternating current. In our experiments analogous observations could be made in the dog, the cat and the pig. Whereas most animals remained quiet some minutes after the current was opened, others became angry and aggressive within one to one and a-half minutes. The cat spits and attacks the approaching hand; it is the same case with the dog, who bellows. The pig yells. They all showed a malicious temper and tried to get away from the table and from the room. These symptoms of anger sometimes disappear after some minutes. However, this is not always the case. For instance, a cat which was subjected to a current of 200 ma. during 10 seconds was dangerous because of its fury as soon as some fifty seconds had elapsed after the experiment, and persisted in this state for about an hour. The animal followed every movement in the room with its eyes from the beginning of excitation; the other animals acted in the same manner. A dog subjected repeatedly to a current of 200 ma. during an afternoon was very angry the next morning. The animal was aggressive, growled and showed its teeth when the kennel was approached. During four days this condition was retained. The dog had been very kind before. Another dog tried to fly from the room fifteen minutes after the current of 175 ma. had been given during ten seconds. The behaviour of some cats deserves attention. As soon as the current was opened they showed polypnœ, with very frequent respiratory movements (90–240 per minute), the tongue hanging out of the mouth. Forty-five seconds after the experiment they presented symptoms of anger, the cornea reflex being positive at this moment.

Discussion and Inferences.

The results ensuing from the experiments described are that the alternating current of 50, 60 or 70 volts, conducted through the skull of the animals in the way indicated by the constructor of the apparatus used, acts as a strong excitation on the cerebro-spinal as well as on the autonomic nervous system. The sensitiveness of the oblongata to this current was demonstrated for the three centres found here—the vasomotor, the respiratory, and the vagal centre. The nystagmus horizontalis of the bulbi must probably be attributed to another centre of the medulla oblongata—the nucleus Deiters.

Owing to this excitatory action on the nervous system, the alternating current is in contrast to the intermittent constant current, which latter paralyses the motoric centres, as Leduc[6] demonstrated. This contrast has also been described recently by Scheminzky[12] in fishes. Scheminzky had previously found[11] that an alternating current was able to bring fishes into a motionless condition which resembled the "electronarcosis" of Leduc, into which these animals can be brought by a constant current. Further investigations[12] made it evident, however, that the effect was an entirely different one if the alternating current was used. In this case the fishes and the tadpoles exhibited general muscle spasms, which rendered any movement whatsoever impossible.

Bikeless and Zbyszewski,[2] investigating the action of weak alternating current (up to 75 ma.), demonstrated its excitatory effect on dogs and rabbits. These authors recorded arterial blood-pressure while the current passed through the skull of the animals in a vertical direction. Nor did they detect any sign that could be regarded as a symptom of stunning or narcosis.

If we now try to understand on what grounds, besides those of practical consideration, veterinary surgeons introduced the alternating current into slaughter-houses in order to stun animals, the following must be kept in mind: (1) The animals subjected to the current exhibit no pupil reaction to light. As it was shown above, this observation may induce false conclusions to be drawn as to the state of consciousness of the animals; and this was the case. (2) The same is the case in a still higher degree with the cornea and conjunctival reflex. Much more value has been attached to this reflex than it deserves, apparently as a sequel to the rôle which it plays in chemical narcosis. Many meat hygienists come to the conclusion that the pigs are unconscious because of the absence of the cornea reflex. So, for instance, Matthiesen and Grüttner,[8] who found the reflex not elicitable during fifteen seconds after the current was stopped. Hobday[4] also concluded that pigs appear to lose consciousness instantaneously as soon as the electrodes were applied, the conjunctival reflex being immediately abolished. This conclusion is not justified and cannot be right. It is clear that the conjunctival reflex, *i.e.*, shutting of the eyelids after the conjunctiva was touched, is only possible provided that the eyelids are free to react. Since the eyelids are now brought into tonic spasms as soon as the circuit is closed the reflex is prevented now. In this condition absence of the reflex does not show anything as regards the condition of the central nervous system. Control of the conjunctiva reflex is as little valid now as the control of the validity of the lock of a nailed door. And after the circuit is opened the cornea reflex remains an unreliable indicator, as the excitation of the sympathetic nervous system, by which the eyelids may be opened (the muscle of Muller), proceeds a long time after the current was broken. In this way closure of the eyelids can be impeded and even prevented, as it was proved by special experiments. So this reflex, of unratable value in animals under chemical narcosis, is not apt at all to the task appointed: to bear the burden of proof as regards the condition of the central nervous system in its entirety. Obviously a negative cornea reflex is of far less importance in judging the state of the central nervous system in animals subjected to the electric current than is the case in the animal under narcosis.

In our experiments the cornea reflex was generally elicitable again three-quarters to one and a-half minutes after the current was stopped. A shorter time was not an exception however. Periods of 20 seconds were pretty frequently found in the cat; once an interval of ten seconds was sufficient. In the dog analogous periods are encountered. In one experiment on the pig the reflex was positive after 20 seconds, in another even after ten seconds. In the goat periods of 20 to 35 seconds are found. It is curious that in the dog and

in the pig the eyelids flickered in a single case before the cornea reflex could be intentionally elicitated, although flickering is a reflex when the animal is under physiological conditions. Probably the movements of the eyelids found here are not reflectory, in contrast to the physiological ones. This view is supported by the experience that they may coincide here with convulsions of the skeletal muscles. It is more probable, therefore, that these movements of the eyelids are convulsions, too, evoked by a convulsion of the muscle orbicularis oculi.

The reports on the use of the "electrolethaler" for humane slaughter of pigs and sheep, given by Symes, L. Hill and Hobday,[13] at the request of Mr. Marsh, the Managing Director of the factory of Messrs. Marsh and Baxter, may not be left undiscussed. These reports are based merely on watching the pigs treated with the current. Some seventy-five animals were subjected to the alternating current from 60 to 70 volts. The joint report concludes that the pigs appear to lose consciousness instantaneously as soon as the tongs were applied, " the conjunctival reflex being abolished immediately." Other arguments not being given, it is sufficient to refer to the comment given above.

All the three individual reports agree with regard to this conclusion. Symes adds that the current produced immediate rigidity in the trunk and limbs. The animals neither gave any sound nor showed any struggle or other sign of preliminary discomfort. We are quite willing to believe this; but we cannot understand how the animals ever could give a sign of discomfort whatever, as the muscles immediately became rigid. Hobday's conclusion, based on the conjunctival reflex being abolished as soon as the current passed, has already been discussed. Hill concludes that the animals lose consciousness very quickly, and bases this on the following consideration : As the eyelid and respiratory centres are thrown out of action by the passage of the current, it is clear that the higher centres subserving consciousness, which are far more sensitive and go out of action first, must be rendered inactive. We regret sincerely that we are not able to agree on this point. The only thing Hill could observe when surveying the animals was that the breathing stopped, the pupils became widely dilated and insensitive to light, the conjunctival reflex was lost. But neither the respiratory centre nor the eye centre are " thrown out of action." It is quite the reverse, their activity was not diminished, but highly increased, as was shown.

These reports therefore cannot contribute towards an understanding of this difficult question as to the painlessness and the unconsciousness of the animals. Neither does the experience of the reporters concerning the sheep, nor Hobday's short report on the application of the current for anæsthetising three pigs before operation. The number of investigations undertaken in order to analyse the effect of the alternating current on animals, with an aim of obtaining insight into the fundamental question, the condition of the animal, is exceedingly small, however great the number of papers on the application of the method may be.

Battelli[1] studied the effect of alternating currents of 120–140 volts in the dog and the monkey. The question as to the animals having lost consciousness

or not was not discussed. A meritorious paper has been given by Clark and Tweed in 1932.[3] These investigators recorded arterial and venous blood-pressure in the sheep while the animal was subjected to an alternating current of 50 volts. The result agrees with ours in so far that both the arterial pressure and the venous pressure rose considerably. Pigs were also treated with the current, while the electrodes were applied on either side of the neck.

The authors are careful in their conclusions. As regards the pigs, they say consciousness is lost at once, " so far as can be judged." As to the sheep, " there is little reason to doubt that consciousness is lost." For, the authors continue, "if consciousness was not lost the animals would make some attempt to regain their feet immediately the current is switched off, and would undoubtedly give vocal evidences of discomfort."

As it seems to us, the behaviour of these animals does not support the conclusion of the authors. Their animals exhibited tonic muscular contractions and the pigs became rigid and immobile, they say. As it was shown before, the increased activity of the motor centres does not stop, however, at the moment the current is stopped, and as the skeletal muscles as well as the muscles of the larynx are involved, it cannot be expected that the animals could react in the way the authors suggest. So this immobility may be regarded as the result of the increased activity of the central nervous system, and not as a sign that could prove its activity to be abolished.

After having studied the literature we must repeat our conclusion, that the picture of animals subjected to the current used is characterised by symptoms of excitation of the central nervous system only. Signs of stunning, exhaustion, or protection are not observed. It is, nevertheless, possible that consciousness and perception of pain, which are not accessible to direct control, are influenced in an opposite way. This is possible, though it cannot be rendered credible either in view of physiological facts or in view of our experiments. This does not conclude that it is impossible that loss of consciousness is evoked by the alternating current used. If the current proceeds long enough, then the arrest of respiration excites asphyxia, and the latter must cause loss of consciousness. For the question here under discussion, this is not of great consequence however, the time wanted being too long. It is even questionable whether such a state would exist at the end of the period the respiratory movements are arrested. Loss of consciousness in consequence of pain brought about by the muscle spasms may also be possible.

Finally, the possibility remains that an interference in the nervous system so crude as it was applied here prompts a condition of strong excitation, combined with general " inhibition," which we cannot further. But by accepting this we are touching a dominion inaccessible for concrete perception, and where exact knowledge fails.

Summary.—The effect of an alternating current was studied in the cat, the dog, the pig, the goat, the rabbit and the calf. The current was obtained from an apparatus constructed for stunning pigs prior to slaughter ; it transformed

the alternating current of the town (fifty periods per second) to 50, 60 or 70 volts. The apparatus corresponds with the "electrolethaler." The current passed through the skull of the animals in transverse direction during the time and in the way as indicated for the apparatus.

Normal and narcotised animals were used. Besides these, thalamus cats were subjected to the current. The results are as follows: (1) The effect in all animals is characterised by a strong excitation of the cerebrospinal and the autonomic nervous systems. Signs of fatigue, exhaustion, paralysis or stunning of the nervous centres, even when using a current lasting up to 100 per cent. longer than it is commonly applied, are not found. Nor are the nervous centres protected by the electric current. (2) There is no point of correspondence with narcosis; fundamental contrasts are proved to exist between these two. (3) The effect of the electric current does not lengthen the duration of narcosis, nor does it complete partial narcosis. It rather works against the latter. (4) Literature is discussed and the conclusions found there are commented on.

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OBSERVATIONS ON ACTIVE IMMUNISATION AGAINST ANTHRAX.

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Introduction.

ACTIVE immunisation of animals against anthrax is the most generally adopted method of attempting the eradication of the disease. The most successful vaccines have consisted of attenuated cultures containing vegetative or spore forms. The results, generally speaking, and particularly with the spore forms, have been good, although some occasional serious losses following vaccination have been reported. In the introduction to his article on anthrax immunity

Quin[1] says: "However efficient our present anthrax vaccines may be, it should always be borne in mind that the wholesale inoculation with live organisms, although attenuated, is far from ideal, since they are directly derived from the dangerously virulent organism, the biology of which is not completely known."

Further, some evidence has been brought forward by Stylianopoulo and Pheloukis[2] to show that the second vaccine of Pasteur can elaborate soluble toxic products capable of causing death. The danger visualised should be diminished with a vaccine such as the Italian "Carbozoo," where the organism is localised at the site of inoculation, and in the absence of a killed vaccine of proved value in the field, the claims of the Italian workers require investigation.

Immunisation of livestock against anthrax is one of the major activities of the Veterinary Service in Cyprus. The number of animals vaccinated annually has increased rapidly during the last ten years. The number of animals vaccinated during the vaccination campaign of 1932 was as follows: Equines, 448; cattle, 229; sheep, 275,701; goats, 168,344; pigs, 90; total, 444,812.

Various types of vaccine have been used during the last few years. From 1921 to 1926, Evans double vaccine was used, 800 animals being vaccinated in 1922, and over 70,000 in 1926. Pasteur single vaccine was then introduced, and was in general use until 1930, when the use of South African vaccine commenced. This has been followed by the use of vaccine prepared locally by the South African method. Generally, the results of vaccination have been more satisfactory after vaccine prepared by the last method was introduced. The work presented here records the results obtained when preparing vaccine by the South African method for field use, and also describes the results of some preliminary experimental tests of an Italian method.

The South African Method.

Introduction.—The South African vaccine is a spore vaccine prepared from attenuated strains. Vaccination with spores has been carried out in various countries for many years, apparently the first attempts being made in Russia, by Cienkowsky, in 1884,[3] and this type of vaccine possesses a decided advantage over vaccine containing vegetative forms in that it retains its potency fairly constantly for several months. A recent communication from South Africa indicates that vaccine prepared at the Onderstepoort laboratory maintains its potency when kept under suitable conditions for nearly twelve months.

After numerous experiments the workers at Onderstepoort determined that a safe vaccine could only be produced by adopting strains of varying degrees of attenuation for the various species of animals. The attenuated strains used are three in number, namely (1) sheep and cattle, (2) goat, (3) equine. Vaccine has only been prepared in Cyprus from the first two strains. Strains already suitably attenuated have been obtained by us from South Africa, and it is therefore not proposed to discuss here the method of attenuation. The fact that we were able to obtained these strains and competent advice from the Onderstepoort workers greatly facilitated vaccine preparation.

Technique of Preparation.—In general, the technique adopted has followed closely that used at Onderstepoort by Viljeon, Curson and Fourie,[4] but the following outline introduces some minor modifications which have been found useful in Cyprus.

(*a*) *Seed Material.*—The attenuated vaccine strains are kept in hermetically-sealed agar tubes in order to avoid contamination and drying-out. They can be kept thus in spore form for many years. It should be noted, however, that it may be necessary, as we found, to pass the strain through a guinea-pig prior to commencing the preparation of a batch of vaccine.

(*b*) *Subcultures.*—Subcultures are made in two broth tubes, which are incubated for forty-eight hours at 37° C.

(*c*) *Purity Test.*—During this period the purity of the culture is ascertained by naked-eye examination, stained smear examination, and subculturing.

(*d*) *Inoculation of Half-litre Broth Flask.*—To avoid contamination later, this flask is fitted with a cotton-wool plug through which pass two glass tubes, one of these being below the plug for two or three inches. This serves for the introduction of the seed material from the tube of broth. The other tube reaches the bottom of the flask, and is bent outside to an angle of 35 degrees. The outside extremity near the mouth of the flask is fitted with a piece of rubber tubing, which is attached to another piece of glass tubing six inches long, with its end drawn out and sealed. After forty-eight hours' cultivation and the usual purity tests, the seed material can be drawn off through this tube.

(*e*) *Inoculation and Incubation of Fernbach Flasks.*—Maximum sporulation requires free exposure to oxygen, and these flasks are used because of the large surface area of agar made available. A thin layer of agar of Ph. 7.6 is poured into the flasks, which are then carefully sterilised. They are then incubated for several days at 37° C., in order to check sterility and to evaporate excessive moisture. The number of flasks necessary naturally varies with the final dilution of the vaccine, but with the strain used here we have obtained over 260,000 sheep doses from thirty-eight flasks. Each flask is now inoculated with enough seed material to moisten the entire surface, and this step must be carried out very carefully under the flame in order to avoid contamination. The flasks are now incubated at 35°–37° C. until sporulation is practically complete. Some of the flasks are examined daily to observe the extent of sporulation. When sporulation has reached 80 to 90 per cent., the flasks are removed from the incubators and placed in cupboards at room temperature for a week.

(*f*) *Preparation of Concentrated Emulsion.*—The growth is washed off by the introduction of a small quantity of saline, and a few glass beads into each Fernbach flask. It has been found convenient to prepare a number of test tubes each containing 8 c.c. of saline, and a dozen glass beads. After sterilisation the contents of a test tube are easily poured into each flask. The spore emulsion so obtained is poured into a two-litre flask of known weight. In order to keep back the beads and any particles of agar, a sterile Buchner's filter is fitted to the mouth

of the flask, and proves to be quite satisfactory. Sterile glycerine is now added, two parts by weight to one of the original emulsion. The final dilutions of the vaccine are made from this flask, but as we find the dilutions can be satisfactorily made with a pipette, the flask is not fitted with tubing.

Technique of Tests of Concentrated Glycerine Spore Emulsion.

The tests and the results to be expected therefrom vary with the original strain. Preliminary tests are carried out on small laboratory animals, and final tests on sheep and goats.

(1) *Preliminary Sheep—Vaccine Test.*—

1 rabbit,	subcutaneously	.1 c.c. emulsion
1 ,,	,,	.01 c.c. emulsion
1 guinea-pig	,,	.01 c.c. emulsion
1 ,,	,,	.001 c.c. emulsion

The vaccine is considered to be safe and of sufficient strength if both guinea-pigs died while both rabbits survived. If only the guinea-pig receiving the larger dose should die, the vaccine would still be strong enough. If both rabbits died, the vaccine should be discarded.

(2) *Preliminary Goat Vaccine Test.*—

1 guinea-pig	subcutaneously	.1 c.c.
1 ,,	,,	.01 c.c.

Both guinea-pigs should survive.

As will be shown later, however, the results of these preliminary tests, while serving as some guide to the strength of the emulsion, must not be considered as final.

(3) *Final Sheep Vaccine Test.*—The final tests should be carried out three to four weeks after the spore emulsion is washed off and mixed with glycerine, but it can be carried out later.

Safety Test.—

Two sheep,	subcutaneously,	20 c.c. each of emulsion
,, ,,	,,	1 c.c. each of emulsion
,, ,,	,,	.1 c.c. each of emulsion
,, ,,	,,	.02 c.c. each of emulsion
,, ,,	,,	.01 c.c. each of emulsion
,, ,,	,,	.005 c.c. each of emulsion

All sheep should survive.

Immunity Test.—This test is carried out on the same animals three weeks later. A recent communication from South Africa indicates that an immunising value sufficient to protect against fifteen sheep, M.L.D., is satisfactory. With a good vaccine at least the sheep receiving 0.01 c.c. of vaccine would be protected. In this case the emulsion would be diluted a hundred times in 60 per cent. sterile glycerine saline. This dilution is made in large sterile flasks suitably fitted to permit of aseptic bottling. We find that an ordinary test-tube filler attached to each flask fits well over the neck of the vaccine bottle in use, and serves as a very efficient hood.

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(4) *Final Goat Vaccine Test.*—A number of goats are inoculated with various dilutions of the concentrated emulsion, and subsequently tested with virulent material as described in the sheep vaccine test. All goats receiving the vaccine emulsion should, of course, survive. The results obtained by the workers at Onderstepoort when vaccinated test goats have subsequently received virulent material have been variable, since it is sometimes found that a goat which had received a small amount of vaccine survived, whereas a goat receiving a larger amount of vaccine has succumbed to the immunity test. The susceptibility and resistance of goats to anthrax in South Africa is evidently a very variable quantity. In making an estimation of the amount of dilution necessary to be made of the concentrated spore emulsion, it is evidently the rule to make the goat vaccine on the weak side, at the same time ignoring an odd death which might occur on test in a goat receiving a large dose of vaccine emulsion, and which one might reasonably have expected to have developed immunity. Generally speaking, the field results obtained in South Africa with vaccine so prepared have been satisfactory.

Virulent Test Material.—In carrying out the immunity test it is, of course, necessary to use a virus of known strength, and since the virulence of such a preparation can diminish with storage, it is essential that the M.L.D. should be frequently redetermined. The virulent material consists of a glycerine spore emulsion prepared in the same way as the vaccine, but, of course, from a highly virulent strain. The M.L.D. is determined by the injection of varying dilutions of the emulsion into sheep.

Sheep Vaccine. Preparation and Testing. Preliminary Batch No. 1. Determination of M.L.D. on Test Material.

Virulent emulsions were prepared from a South African strain (1 c.c. = 1,000 M.L.D.), and tested on sheep on four occasions. It appeared that the sheep we used had a considerable resistance to this strain, since it was only after passage of the strain through a sheep that we obtained a sufficiently virulent emulsion, so that 0.001 c.c. was the minimum lethal dose.

Vaccine Emulsion.—Three small quantities of vaccine emulsion were prepared, but in each case the test carried out on guinea-pigs and rabbits indicated that the emulsion was not of sufficient potency to justify further tests on sheep. The strain was therefore passed through a guinea-pig and isolated afresh from the heart blood. A spore emulsion was then prepared, which gave very satisfactory results when tested on laboratory animals.

RESULTS.

Animal.	Route	Inoculation Strain 30	Result
Guinea-pig 1	Subcutaneous	0.001 c.c.	Died, anthrax, forty-three hours (about)
Guinea-pig 2	Subcutaneous	0.01 c.c.	Died, anthrax, forty-three hours (about)
Rabbit 1	Subcutaneous	0.01 c.c.	Lived
Rabbit 2	Subcutaneous	0.1 c.c.	Lived

This vaccine emulsion was then tested on sheep. The virus was given twenty-two days subsequent to vaccination, and the results are shown below :

Animal	Vaccine Emulsion	Reaction	Virus	Result
Sheep No. 32	5 c.c.	Died in 7 days, not anthrax	—	—
Sheep No. 23	5 c.c.	Temperature	500 m.l.d.	Lived
Sheep No. 30	0.1 c.c.	Temperature	500 m.l.d.	Lived
Sheep No. 31	0.1 c.c.	Temperature	500 m.l.d.	Lived
Sheep No. 28	0.01 c.c.	*Nil*	500 m.l.d.	Lived
Sheep No. 29	0.01 c.c.	Temperature	500 m.l.d.	Lived
Sheep No. 26	0.005	Died in 15 days, not anthrax	—	—
Sheep No. 27	0.005 c.c.	Died in 7 days, not anthrax	—	—
Sheep No. 47	Control	—	500 m.l.d.	Died anthrax 46 hours

The three deaths subsequent to vaccination were unfortunate, but could not be attributed to the vaccination. Two out of five unvaccinated sheep of the same flock died during the same period, all being heavily infected with Moniezia expansa. 0.01 c.c. of the emulsion, therefore, protected against 500 M.L.D. of virus, and thus the emulsion was diluted a hundred times with 50 per cent. glycerine saline to make the field dose of vaccine 1 c.c. Two thousand nine hundred c.c. of this vaccine were prepared. Prior to issue a test vaccination of twenty-five sheep in the field gave satisfactory results.

Sheep Vaccine—Batch 2 : Minimum Lethal Dose of Test Material.

The virulent strain was isolated afresh from sheep 65, and, adopting the usual technique, a glycerinised spore emulsion was prepared. Sheep were inoculated with the following results :

Animal	Dose	Result
2 sheep	0.01 c.c. subcutaneously	Temperature reaction
2 sheep	0.1 c.c. subcutaneously	Temperature reaction

The emulsion was therefore not of sufficient strength for practical purposes. A large dose of this strain was administered to sheep 64, which subsequently died. A pure culture of anthrax was obtained from the spleen, and a spore emulsion was prepared from this culture. From the five Fernbach flasks which were inoculated, 34 grams of saline emulsion were obtained. Double this weight of sterile glycerine was added, and the M.L.D. test gave the following results :

Animal	Dose	Result
Sheep 62	0.01 c.c. subcutaneously	Temperature reaction
Sheep 63	0.01 c.c. subcutaneously	Temperature reaction
Sheep 59	0.05 c.c. subcutaneously	Died seventh day, anthrax
Sheep 60	0.1 c.c. subcutaneously	Died second day, anthrax
Sheep 61	0.1 c.c. subcutaneously	Died fourth day, anthrax

One c.c., therefore, equals approximately 20 M.L.D. This indicated only a moderate virulence, but we were able to make practical use of the emulsion.

Vaccine Strain 30.—Forty flasks were used in the preparation of vaccine. With our facilities, sterilisation of flasks and preparation of media take about seven days. The vaccine strain No. 30 was isolated afresh from the heart blood of a guinea-pig, and a spore emulsion was prepared from the Fernbach flasks,

adopting the usual technique. The Fernbach flasks were examined frequently to obviate the risk of over-incubation, and sporulation was found to be almost complete on the third day. The flasks were then placed in the cupboards for seven days. A series of test tubes were prepared, each containing 8 c.c. of saline and about a dozen beads. After sterilisation of the series the contents of one tube were emptied into each flask. The growth was then washed off and shaken. The beads were caught by passing the emulsion through a sterile Buchner's funnel. Two hundred and fifty-five grams of saline emulsion were obtained from thirty-eight flasks; 510 grams of sterile glycerine were added to this emulsion; 675 c.c. in volume of the glycerine emulsion were thus obtained. The test on laboratory animals was carried out two days later, and resulted as follows:

Rabbit	0.1 c.c. subcutaneously	Lived
Rabbit	0.01 c.c. subcutaneously	Lived
Guinea-pig	0.1 c.c. subcutaneously	Died second day, anthrax
Guinea-pig	0.01 c.c. subcutaneously	Died third day, anthrax
Guinea-pig	0.001 c.c. subcutaneously	Died third day, anthrax

Twenty-two days after this test, a series of sheep were vaccinated, and twenty-one days later they received virus with the following results:

Animal. Sheep	Vaccine emulsion	Reaction	Virus	Reaction
No. 76	20 c.c.	Temp. 2 days	40 m.l.d.	*Nil*; lived
No. 66	12 c.c.	Temp. 2 days	40 m.l.d.	Temperature 2 days; lived
No. 69	0. c.c.	Temp. 1 day	40 m.l.d.	Temperature; slight swelling at site; lived
No. 75	0.1 c.c.	Temp. 4 days	40 m.l.d.	Temperature; slight swelling at site; lived
No. 67	0.02 c.c.	Temp. 2 days	40 m.l.d.	*Nil*; lived
No. 68	0.02 c.c.	Temp. 2 days	40 m.l.d.	Temperature 3 days; lived
No. 77	0.01 c.c.	Temp. 1 day	40 m.l.d.	*Nil*; lived
No. 72	0.01 c.c.	Temp. 1 day	40 m.l.d.	Temperature; slighly lame; lived
No. 71	0.005 c.c.	Temp. 4 days	40 m.l.d.	Temperature; lived
No. 74	0.005 c.c.	Temp. 1 day	40 m.l.d.	Temperature; lived
No. 70	Control	—	40 m.l.d.	Temperature 8 days; lived
No. 85	Control	—	40 m.l.d.	Died anthrax, 3½ days
No. 86	Control	—	40 m.l.d.	Died anthrax, 3½ days

Temp. = Temperature reaction.

Controls 85 and 86 received their inoculation three days after the other sheep. They were included because control 70, which was an old sheep, appeared to be offering some resistance. All the sheep were inoculated from the same flask of virulent emulsion. Both sheep which received 0.005 c.c. of vaccine emulsion survived the test inoculation, and this emulsion was therefore diluted 200 times in 50 per cent. glycerine saline. The dilutions were carried out in two litre flasks suitably fitted for subsequent bottling of the vaccine. Each flask contained 1,500 c.c. of sterile 50 per cent. glycerine-saline, to which 7.5 c.c. of the vaccine emulsion was added with a pipette. Ninety large flasks of vaccine were obtained, and allowing one flask for wastage, this equalled approximately 267,000 sheep doses of 0.5 c.c. Since the field dose for sheep is 0.5 c.c., sheep No. 76, which received 20 c.c. of the concentrated spore emulsion, received a dose representing 8,000 vaccine doses, a good indication of the safety of this vaccine. Successful tests were carried out on twenty sheep in the field, and twenty-five sheep (five

Merinos, ten cross-bred, ten native) at Athalassa Stud Farm. The post-vaccination temperatures of ten of these sheep were recorded, and in seven cases there was an increase in temperature lasting not longer than three days. A further flock of 200 sheep was vaccinated at the Government Stud Farm prior to the issue of the batch for general field use.

Sheep Vaccine—Batch 3.

Four guinea-pigs were inoculated with varying amounts of cultures of strain 30 without effect. A guinea-pig was then inoculated with 10 c.c. of vaccine 30, batch 1, and died in twenty-four hours from anthrax. One would have expected the organism maintained by subculture on media to have been more potent than the spores maintained in glycerine. The organism was thus isolated afresh, and from this culture a glycerinised emulsion was prepared in the usual way. Sporulation was nearly complete in approximately 3½ days, and from thirty-eight Fernbach flasks, 283 grams of saline emulsion were obtained. Five hundred and sixty-six grams of sterile glycerine were than added. The total amount of concentrated spore emulsion equalled about 690 c.c.

Tests on Laboratory Animals.—The first test was carried out forty-eight hours after the preparation of glycerinised emulsion was completed :

Guinea-pig inoculated subcutaneously with 0.1 c.c.
Guinea-pig inoculated subcutaneously with 0.01 c.c.
Guinea-pig inoculated subcutaneously with 0.001 c.c.
Rabbit inoculated subcutaneously with 0.01 c.c.
Rabbit inoculated subcutaneously with 0.1 c.c.

Two guinea-pigs died within forty-eight hours, and a third within seventy-two hours, from anthrax. One rabbit died on the fifth day, and the second on the seventh day from anthrax. The same strain (30) had been used previously in the preparation of Batch 2, and both rabbits then survived the test. It was therefore thought advisable to repeat the test after the glycerinised emulsion had been standing for a longer period, in order that the vegetative forms present should be reduced. Fourteen days after the emulsion was prepared the test was therefore repeated with more satisfactory results :

Guinea-pig inoculated subcutaneously with 0.001 c.c.
Guinea-pig inoculated subcutaneously with 0.01 c.c.
Rabbit inoculated subcutaneously with 0.01 c.c.
Rabbit inoculated subcutaneously with 0.1 c.c.

Both guinea-pigs died from anthrax, whereas both rabbits survived. We therefore felt justified in proceeding with the tests on sheep.

Minimum Lethal Dose.—In order to carry out the test on sheep, it is necessary to redetermine the M.L.D. of the virulent material a short time before the test. An attempt was first made to increase the virulence of our strain by passage through sheep. Sheep No. 27 received 2 c.c. broth culture of strain 64, but did not react. Sheep No. 5 received subcutaneously an emulsion of an agar slant. She died

within forty-eight hours, and pure cultures were obtained from an ear vein. From these cultures sheep No. 7 received 7 c.c. mixed broth culture and agar slant emulsion. She had a temperature of 107° F. for two days, when she received a further 5 c.c. of broth culture. She died the following day, and a pure culture was obtained from the spleen. From the cultures isolated from sheep No. 7 six Fernbach flasks were sown. Three and a-half days' incubation was enough to ensure satisfactory sporulation. Fifty-seven grams of saline emulsion were obtained, and double this weight of sterile glycerine was added. The following are the results of tests carried out on sheep a few days later :

Animal	Dose	Result
Sheep No. 23	Subcutaneously 5 c.c. 1 in 10	Died anthrax third day
Sheep No. 21	Subcutaneously 5 c.c. 1 in 10	Temperature three days ; survived
Sheep No. 13	Subcutaneously 0.1 c.c. 1 in 100	Died anthrax second day
Sheep No. 25	Subcutaneously 0.1 c.c. 1 in 100	Died anthrax third day
Sheep No. 3	Subcutaneously 0.1 c.c. 1 in 1,000	Temperature seven days : survived
Sheep No. 22	Subcutaneously 0.1 c.c. 1 in 1,000	Temperature three days ; survived

One c.c. of the emulsion was therefore taken to represent 100 m.l.d. for sheep. It will be shown later that when goats were inoculated with this emulsion they proved to be much more susceptible than sheep.

Result of Vaccine Test on Sheep (Batch 3).—The sheep received the glycerinised vaccine emulsion thirty-seven days subsequent to its preparation :

Animal : Sheep	Vaccine dose	Reaction	Interval	Virus	Result
No. 20	20 c.c.	Temp. six days, swelling at site	21 days	50 m.l.d.	Temperature 1 day
No. 19	9 c.c.	Temp. 2 days	21 days	50 m.l.d.	Temperature 1 day
No. 1	0.1 c.c.	Temp. 2 days	21 days	50 m.l.d.	Temperature 3 days
No. 4	0.1 c.c.	Temp. 1 day	21 days	50 m.l.d.	Temperature 3 days
No. 6	0.02 c.c.	Temp. 1 day	21 days	50 m.l.d.	Temperature 1 day
No. 18	0.02 c.c.	Temp. 1 day	21 days	50 m.l.d.	Temperature 2 days
No. 8	0.01 c.c.	Temp. 1 day	21 days	50 m.l.d.	Temperature 2 days
No. 11	0.01 c.c.	Temp. 2 days	21 days	50 m.l.d.	Temperature 3 days
No. 12	0.005 c.c.	Temp. 2 days	21 days	50 m.l.d.	Temperature 3 days
No. 14	0.005 c.c.	Temp. 1 day	21 days	50 m.l.d.	Temperature 1 day
No. 26	Control	—	21 days	50 m.l.d.	Died anthrax fourth day.

Temp. = Temperature reaction.

No. 20, which received a dose representing 8,000 doses of the dilute vaccine, developed a marked swelling at the site of inoculation that extended down the hind leg on that side. The swelling disappeared in about eight days. On the morning and evening of the fourth day, after receiving vaccine emulsion, all ten vaccinated sheep exhibited a slight increase in temperature which had fallen again by the next day. Since both sheep which had received 0.005 c.c. of vaccine emulsion survived the subsequent test inoculation, the emulsion was diluted 200 times in 60 per cent. glycerine saline. The dilutions were made into ninety two-litre flasks, each containing 1,500 c.c. glycerine saline, and suitably fitted for bottling. Allowing for a wastage of two litres during the bottling process, the total amount of vaccine prepared equalled 133,000 c.c., or 266,000, sheep doses.

Approximately 100 small bottles of vaccine are obtained from each flask, and each bottle receives a number corresponding with the number on the flask. During the preliminary field trials this facilitates checking the contents of each flask.

Goat Vaccine—Preparation and Testing. Determination of Minimum Lethal Dose of Virulent Emulsion.

The same virulent emulsion that was used in the testing of sheep vaccine, batch 3, was tested on goats. Both sheep which had received 0.001 c.c. had survived.

Animal	Dose	Result
Goat No. 12	0.001 c.c.	Died anthrax third day
Goat No. 13	0.001 c.c.	Died anthrax fourth day
Goat No. 15	0.0002 c.c.	Died anthrax fifth day
Goat No. 10	0.0001 c.c.	Died anthrax fourth day
Goat No. 11	0.00001 c.c.	Lived.

Our field reports indicate that the disease in nature is of considerably more frequent occurrence in goats than sheep, and the above test indicates that they are considerably more susceptible to artificial inoculation.

Preparation of Goat Vaccine.—The strain used was from a bottle of South African Goat Vaccine, No. 34. This vaccine had been used in the vaccination campaign of 1932 with good results. The following guinea-pig inoculations were carried out:

Guinea-pig 1 received 10 c.c. Vaccine 34. Died anthrax fourth day
Guinea-pig 2 received 15 c.c. Broth Culture 34. Died anthrax fourth day
Guinea-pig 3 received 5 c.c. Broth Culture 34. Lived

Pure cultures were obtained from the first guinea-pig. Cultures on Fernbach flasks were then obtained in the usual way.

After four and a-half days' incubation sporulation was well advanced, and the flasks were removed from the incubators. A week later the growth was washed off by adding 9 c.c. sterile saline and a number of sterile beads to each flask. This batch of flasks had become very dry, and the amount of concentrated emulsion obtained was less than usual. Double the weight of glycerine was added, and the total volume was then approximately 450 c.c. Eight days subsequently the material was tested on guinea-pigs with the following results:

Guinea-pig 1 received 0.001 c.c. Died eighth day, anthrax.
Guinea-pig 2 received 0.01 c.c. Died third day, anthrax.
Guinea-pig 3 received 0.1 c.c. Died third day, anthrax.

Sixteen days after preparation the material was tested on two guinea-pigs and two goats, with the following results:

Guinea-pig 1 received 0.001 c.c. Died fifth day, anthrax.
Guinea-pig 2 received 0.001 c.c. Died seventh day, anthrax.
Goat 45 received 0.01 c.c. Lived.
Goat 44 received 0.01 c.c. Lived.

The South African reports indicate that at least the guinea-pig receiving the smallest dose should survive. However, not quite all the vegetative forms had

disappeared from the emulsion when the guinea-pig tests were carried out, and it was possible that these may have influenced the results. In view also of the fact that the vaccine was required at an early date, the test on goats was carried out immediately.

Test on Goats.—The safety test on goats was carried out twenty-four days after preparation of the concentrated glycerine emulsion, except goats 44 and 45, in which the time was sixteen days. The subsequent virulent test consisted of ten sheep M.L.D., or approximately 1,000 goats M.L.D. Number 44, which died, was a small Maltese kid. One goat which had received 0.004 c.c. vaccine died, whereas the other receiving the same amount, and two which had received 0.002 c.c., survived.

The general results indicated that the vaccine was a fairly strong one, and at the same time perfectly safe for the eighteen goats tested. The concentrated glycerine emulsion was diluted 250 times, 6 c.c. being added to each flask of 1,500 c.c. 60 per cent. glycerine saline.

RESULTS.

Animal	Vaccine safety Dose	Reaction	Interval	Virus M.L.D.	Result
Goat 37	10 c.c.	Temp. one day	21 days	10 sheep	Temp. 1 day
Goat 26	5 c.c.	Temp. one day	21 days	10 sheep	Temp. 1 day
Goat 29	1 c.c.	*Nil*	21 days	10 sheep	Temp. 1 day and 5th day
Goat 38	1 c.c.	Temp. two days	21 days	10 sheep	Temp. 2 days and 6th day
Goat 32	0.1 c.c.	Temp. two days	21 days	10 sheep	Temp. 2 days and 5th day
Goat 34	0.1 c.c.	*Nil*	21 days	10 sheep	Temp. 2 days
Goat 28	0.05 c.c.	*Nil*	21 days	10 sheep	Temp. 2 days
Goat 24	0.05 c.c.	Temp. one day	21 days	10 sheep	Temp. 6 days
Goat 30	0.02 c.c.	*Nil*	21 days	10 sheep	Temp. 6 days
Goat 27	0.02 c.c.	*Nil*	21 days	10 sheep	Temp 5 days
Goat 33	0.01 c.c.	*Nil*	21 days	10 sheep	Temp. 2 days
Goat 36	0.01 c.c.	*Nil*	21 days	10 sheep	Temp 2 days.
Goat 44	0.01 c.c.	*Nil*	30 days	10 sheep	Died second day, anthrax
Goat 45	0.01 c.c.	*Nil*	30 days	10 sheep	Temp. 1 day
Goat 23	0.004 c.c.	*Nil*	21 days	10 sheep	Temp. 2 days and 4th day
Goat 35	0.004 c.c.	*Nil*	21 days	10 sheep	Died third day, anthrax
Goat 31	0.002 c.c.	*Nil*	21 days	10 sheep	Temp. 5 days
Goat 25	0.002 c.c.	*Nil*	21 days	10 sheep	Temp. 2 days
Goat 48	Control	—	21 days	10 sheep	Died third day, anthrax

Temp. = Temperature reaction.

On dilution of the concentrated spore emulsion, 78 large flasks of vaccine were obtained. Allowing one flask for wastage during bottling, the total amount of vaccine prepared equals 115,500 c.c., or 231,000 goat doses. Prior to general issue of the vaccine a successful trial was carried out in the field on 373 goats and 25 kids. The fact that this batch of vaccine has been safely used under field conditions indicates that the early guinea-pig test is not always a reliable guide as to the safety of any given batch, and that in spite of an unfavourable guinea-pig test it might be advisable to proceed with the test on goats.

The Italian Method.

Introduction.—A new vaccine called " Carbozoo " has recently been extensively used in Italy. It is prepared by the Institut Sieroterapico of Milan. Staub[6]

recently carried out two experiments on rabbits to compare the immunity produced by saponin plus culture with that produced by intradermal inoculation of attenuated culture. In Experiment 1, the saponin vaccine used was supplied by M. Hrusker.

Results.—(*a*) Eight rabbits each received subcutaneously 0.25 c.c. Two died from anthrax, the others exhibiting large scars at the site of inoculation that had not healed after six weeks. (*b*) Eight rabbits each received two inoculations (interval twelve days) intradermally of two unequally attenuated cultures. Two died after the second inoculation. The twelve surviving rabbits were tested after two months with 7,000 spores of virulent culture. All rabbits from group A died. In group B three lived and three died.

In the second experiment the vaccine prepared by Staub consisted of a thousand million spores, plus 6 c.c. of a 10 per cent. solution of saponin.

Results.—(*a*) Eight rabbits received 3/8 c.c. sapovaccine. Seven died when tested two months later with a dose of 700 spores. (*b*) Eight rabbits received attenuated cultures. Two died when subsequently tested.

We have been in communication with the Director of the Italian Institute and with the originator of this method of vaccination, but accurate details of the method have not been obtainable owing to the commercial nature of the Institute. However, Professor Belfonti, the Director of the Institute, kindly supplied me with a sample of " Carbozoo " and some literature on the subject.

Referring to the theoretical aspect of this type of vaccine, Professor Muzzuchi, the originator of " Carbozoo," says: " La presupposition theorique suivant laquelle la substance additionnée au germe provoquerait la formation d'une barrière empechant la diffusion de l'infection, et cette reaction de l'organisme dans son essence intime ne serait autre chose qu'une reaction exaltée du type de celle que l'on observe au cours de l'infection charbonneuse, trouve ici sa confirmation la plus suggestive."[5]

The theory is that the addition of saponin creates a defensive barrier which prevents the diffusion of the culture organism throughout the body. At the same time the antigenic properties of the mixture are retained.

CARBOZOO TEST.

Animal	Vaccine	Reaction	Virus	Result
Sheep 83	1/8 c.c., tail	Temp. one day; site sensitive three days	40 m.l.d.	Lived
Sheep 84	1/8 c.c., neck	Temp. one day; site sensitive three days	40 m.l.d.	Lived
Kid 3	1/8 c.c., tail.	Temp. two days; pigeon's-egg swelling at site	10 m.l.d.	Lived

The virus was given twenty-two days subsequent to vaccination.

Observations on Saponin plus Culture (10 *per cent. Saponin*).—Mazzuchi (*loc. cit.*) gives the percentage of saponin as being 10 per cent. He says: " L'addition de bacilles et de spores du charbon virulents, à ce mélange, forme ce complexus vaccinal qui prend le nom de ' Carbozoo ' ; la quantité de saponine qui est presente dans le mélange peut aussi corresponde au 10 per cent. et elle est bien

tolerée même à la dose de 2 c.c. administrée par la voie sous-cutanée aux animaux de taille moyenne et grand."

For our experiments a small quantity of saponium purissimum album was obtained by us from England. From a 50 per cent. solution of saponin, a 10 per cent. solution was made in a forty-eight-hour broth culture of virulent anthrax

TEST ON LABORATORY ANIMALS.

Animal	Inoculum	Result
White rat	Subcutaneously 2 c.c. Sapovaccine	Died in about 24 hours; local reaction severe; blood smear negative
White rat	Subcutaneously 2 c.c. Sapovaccine	Died third day; blood smear negative
White rat	Subcutaneously 2 c.c. Virulent anthrax	Died fourth day, anthrax
White rat	Subcutaneously 5 c.c. Sapovaccine	Died fifth day; reaction localised
White rat	Subcutaneously 5 c.c. Sapovaccine	Died fifth day; reaction localised.
White rat	Subcutaneously 5 c.c. Virulent anthrax	Died seventh day
Guinea-pig	Subcutaneously 5 c.c. Sapovaccine	Died fifth day, anthrax
Guinea-pig	Subcutaneously 5 c.c. Virulent anthrax	Died fifth day, anthrax

The presence of saponin in the mixture appeared to localise in some cases the hæmorrhagic œdema associated with this type of inoculation. The local reactions were very severe.

Reaction of Sheep to Above Mixture.—Anthrax-immune sheep No. 62 received 2 c.c. subcutaneously in the side of the neck. The following day the skin at the site appeared thickened, tense, hot and red. In a few days a white area, the size of a shilling and surrounded by an inflammatory zone, was formed. The white area finally broke down, and a considerable quantity of pus escaped. No bacteria were present in this pus. Sheep 73 received 1 c.c. of the above mixture in the tail. The local reaction was similar to the previous case. After seventeen days she received 40 M.L.D. of virulent anthrax. Some temperature reaction occurred, and the respirations were laboured for two days, but the animal survived. With our saponin, it is evident that 10 per cent. in the mixture causes a very severe local reaction.

Spore Emulsion plus Saponin to 2 per cent.—Professor Belfonti,[7] Director of the Institute at Milan, has informed me that the amount of saponin (Merck) may be 2 per cent. A virulent anthrax strain was grown on solid media in a Fernbach flask until sporulation was well advanced. This was washed off with a few sterile glass beads and 10 c.c. of sterile saline; 200 c.c. of sterile saline were then added. This was called emulsion "A." A dilution of 1 in 10 of "A" in sterile saline was made in two tubes, and saponin added to one tube to make 2 per cent. A rabbit and a rat received 0.5 c.c. of the virulent material subcutaneously. The rabbit died from anthrax in three days. The rat survived. The tube containing the 2 per cent. saponin was allowed to stand at a room temperature for a few days; 0.5 c.c. was then give subcutaneously to a rabbit, which survived. A dilution of 1 in 100 of "A" in sterile saline was made in two tubes, and saponin added to 2 per cent. in one tube. A rabbit received 0.3 c.c. virulent material,

and died of anthrax in three days. A rabbit received 0.3 c.c. virulent material, plus 2 per cent. saponin, and survived.

A dilution of 1 in 1,000 of " A " in sterile saline was made, and saponin added to 2 per cent. It was allowed to remain at room temperature for four days prior to inoculating sheep 91 and goat 5, and sixteen days prior to testing sheep 92 and goat 3.

Animal	Vaccine	Reaction	Virus date	Result
Sheep 92	0.2 c.c.	Swelling size of marble	12 days after vaccine	Died anthrax in 3 days
Goat 3	0.2 c.c.	Small abscess	12 days after vaccine	Temperature reaction
Sheep 91	0.5 c.c.	Small abscess	25 days after vaccine	Slight temperature
Goat 5	0.5 c.c.	Small abscess	25 days after vaccine	Slight temperature
Goat 6	Control	Small abscess	—	Died anthrax in 2½ days

The virus given to each of the above animals consisted of 40 M.L.D., and was from the same flask as that used when testing out sheep vaccine this year. Twenty-five days after receiving 0.5 c.c. of vaccine the sheep and goat appeared to have developed a satisfactory immunity. The local reaction was, however, on the severe side, and further experiments using a smaller percentage of saponin are indicated. It is probable that the degree of dilution of " A " would not be responsible for the lack of pathogenicity of the sapovaccine, and therefore that the saponin doses prevent the invasion of the body by the organism.

Sapo-Vaccine (*Saponin to* 1 *per cent.*).—A Fernbach flask inoculated from virulent strain 64 was incubated for five days, and then placed at room temperature for a week to ensure maximum sporulation. Ten c.c. of sterile saline and a few sterile glass beads were now added, and the growth washed into a small flask containing 200 c.c. of sterile saline. This is called emulsion " A." A small quantity of a 1 in 1,000 dilution of " A " was made, and saponin added to 1 per cent.

INOCULATIONS

After 7 *days* : One rabbit received 0.5 c.c. 1–1,000 " A. "
One rabbit received 0.5 c.c. 1–1,000 " A," plus 1 per cent. saponin.
Both rabbits survived.

After 18 *days* :

Animal	Vaccine	Interval	Virus	Result
Lamb 130	0.5 c.c.	21 days	20 sheep m.l.d.	Lived
Lamb 125	0.25 c.c.	21 days	20 sheep m.l.d.	Lived
Goat 46	0.5 c.c.	21 days	20 sheet m.l.d.	Died anthrax third day
Goat 47	0.25 c.c.	21 days	20 sheep m.l.d.	Died anthrax second day
Goat 43	Control	—	20 sheep m.l.d.	Died anthrax third day

The two lambs and two goats were vaccinated subcutaneously under the tail, and in all cases a severe reaction resulted, a small abscess forming which ultimately burst. They represented typical saponin reactions which have been described previously. A dilution of 1 per cent. saponin is therefore unsuitable for tail inoculations. The two goats could not withstand twenty sheep M.L.D.

Sapo-Vaccine, 1 *per cent. and* 0.2 *per cent.*—This experiment was designed to test the effect of 1 per cent. and 0.2 per cent. saponin in this vaccine when inoculated subcutaneously in the side of the neck. A Fernbach flask was inocu-

lated with virulent strain No. 64, and after five days' incubation and eight days at room temperature, the material was washed off with 10 c.c. sterile saline into 200 c.c. sterile saline. This was called material " A." It was found that 0.5 c.c. of 1 in 1,000 " A " failed to kill when administered to two goats. Another goat was inoculated with 0.5 c.c. of a 1 in 100 dilution of " A " and died from anthrax. This was the dilution used in vaccine preparation. To a portion of 1 in 100 " A,"saponin was added to 1 per cent., and to another portion saponin was added to 0.2 per cent.

Results of Inoculations.

One per cent. Sapo-Vaccine (eight days old) :

Animal	Dose	Interval	Virus	Result
Goat 57	0.5 c.c.	29 days	10 m.l.d.	Lived
Goat 55	0.25 c.c.	29 days	10 m.l.d.	Lived
Goat 60	0.25 c.c.	29 days	10 m.l.d.	Lived

Although the localisation of the anthrax organism was proved, and the immunity produced was satisfactory, the local reaction caused by the saponin in each of the above cases was too severe to permit of the vaccine being used in the field. Swellings about as large as a pigeon's egg occurred, which did not burst, but slowly resolved. It is probable that with the special saponin " Merck " used by the Italians these local swellings can be avoided.

0.2 *per cent. Sapo-Vaccine (seventeen days old)* :

Animal	Dose	Interval	Virus	Result
Goat 56	0.5 c.c.	19 days	10 m.l.d.	Lived
Goat 58	0.25 c.c.			Died from anthrax

Both the above goats exhibited some local reaction at the site of inoculation sufficient to render the use of the vaccine in the field impractical. It seems evident that the type of saponin purissimum album used in the above experiments causes a more severe local reaction to that used in a strength of 10 per cent. by Italian and other workers. It should, however, be noted that one kid inoculated with " Carbozoo " gave an unsatisfactory local reaction. The death of goat No. 58 must be attributed to the reduction in the percentages of saponin in the mixture, or to the marked susceptibility which some goats are known to exhibit to anthrax.

Discussion and Conclusions.

It has been possible in a small-staffed laboratory to prepare a spore vaccine by the South African method sufficient to inoculate approximately 400,000 animals per annum. The safety and efficiency of the sheep vaccine has been demonstrated in laboratory experiments, and its safety proved in the field. It is difficult to assess accurately the value of the field inoculation in the control of the disease, but it is reasonable to presume that the immunity produced is satisfactory.

The immunity produced by the goat vaccine and demonstrated by resistance to test inoculations is not quite so satisfactory, and the very strong susceptibility

shown by some goats must be an important consideration. The results of field inoculations have, however, been good. Very rarely have outbreaks of anthrax occurred a few days subsequent to vaccination, and it is reasonable to presume that the outbreaks are natural, and in no way due to vaccination, particularly when some of the vaccinations are carried out when the summer season, in which the majority of anthrax outbreaks are recorded, is far advanced.

The technique of the preparation of this vaccine is rather elaborate, and a great disadvantage to a small laboratory is the length of time required for its preparation, that is, before one can be sure that the vaccine is satisfactory as to safety and potency. With somewhat limited resources, it takes us about ten weeks from the commencement of preparation of a batch of vaccine until the batch has passed its final tests for safety and potency.

The results of the preliminary tests carried out on guinea-pigs and rabbits are of very doubtful value, particularly since they can be so influenced by variations in the completeness of sporulation. It means that in the absence of complete sporulation, when the concentrated glycerine emulsion is first prepared, the number of vegetative forms present may be sufficient to kill guinea-pigs or rabbits; while if the material is kept a few weeks prior to the test, the glycerine has probably reduced the number or potency of the vegetative forms present, so that, when tested, quite a different result is obtained. This is clearly shown in the preparation of our sheep vaccine Batch 3. At the first test both rabbits died, while fourteen days later at a further test two rabbits survived, and subsequent tests on sheep were satisfactory. Similarly, with the goat vaccine, we found that sixteen days after preparation of concentrated glycerine emulsion, doses of 0.001 c.c. still killed guinea-pigs. After a further ten days the test on goats was carried out with satisfactory results.

From the results of our tests with the virulent strain obtained from South Africa, it would appear that Cyprus sheep offer comparatively a considerable resistance to artificial infection. In preparing virulent material for Batch 3, after passage of the strain through two sheep, the minimum lethal dose was only 0.01 c.c., whereas the original material represented a M.L.D. for South African sheep of 1,000 per c.c. The much greater susceptibility of goats to artificial infection is shown by the fact that when the above virulent material was tested on goats the minimum lethal dose was 0.0001 c.c.

In preparing three batches of sheep vaccine, twenty-eight sheep were inoculated with varying quantities of concentrated spore emulsion. Three of these sheep died, but their deaths could not be attributed to the inoculation. The inoculations were followed in most cases by temperature reactions lasting a few days. All vaccinated sheep resisted subsequent test inoculations of virulent material, while four out of five controls died. These batches of vaccine supplied a total of 538,800 sheep doses, which have been used in the field with satisfactory results.

As the South African authorities have pointed out, the results of tests on goats are not so uniform. All our goats survived the vaccine emulsion, and all but two

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survived the subsequent test inoculation. However, although two goats that received 0.002 c.c. of vaccine emulsion survived the test inoculation, one which received 0.004 c.c. and one which received 0.01 c.c. died. These results can be attributed to the relatively great susceptibility of some goats. Vaccine prepared from the above concentrated emulsion yielded 231,000 goat doses, which have given satisfactory results when used in the field.

While the results of the use of anthrax vaccine prepared by the South African method have so far been satisfactory in our hands, in some countries unaccountable accidents have occurred. This fact, and the length of time required to prepare a batch of vaccine, led one to explore the possibility of obtaining a safer and more expeditiously prepared vaccine. The results so far obtained by us with experiments on sapovaccine have not reached the stage to justify tests in the field. They do, however, furnish a useful basis upon which further work can be carried out.

The originators of "Carbozoo," the Italian vaccine, and other Continental workers who have used this vaccine on a large scale in the field, claim that it is very successful. Others who have attempted to produce a vaccine of this nature, that is, culture plus saponin, have not obtained such satisfactory results. A small test of "Carbozoo" was satisfactory, except that the local reaction in the kid was rather too severe for practical purposes. Throughout our experiments with saponin plus culture, a saponin purissimum album was used, although it was not the preparation "Merck" used by the originators of "Carbozoo." Although various percentages of saponin have been tried in the mixture, in all cases the local reactions have been too severe to permit of any field inoculations being carried out.

White rats, guinea-pigs and rabbits which have been inoculated subcutaneously have all shown severe local reactions, some dying as a result of the inoculation. These severe local reactions occurred in sheep and goats even when the percentage of saponin was reduced to 1, and in goats to 0.2, the inoculations being carried out subcutaneously either on the under side of the tail or at the side of the neck.

A total of five sheep and nine goats have been inoculated with the different mixtures of saponin and culture, and then subjected to inoculation of known virulent material. One of the sheep died from anthrax, the interval between vaccination and virus inoculation being twelve days, which was possibly not sufficient to allow for the full development of immunity. Two of the goats died after receiving a test inoculation of 20 sheep M.L.D. This was twice the dose given in the test of goat vaccine prepared by the South African method. One goat died after receiving vaccine only, the percentage of saponin present in the mixture in this case being only 0.2.

The Italian method may prove to be a more simple and rapid method of preparing anthrax vaccine, and the above results at least justify the carrying out

of further work on the standardisation of the dose, and the elimination of the local reaction.

Appreciation.

I wish to record my sincere thanks to the Director of Veterinary Services, Onderstepoort, South Africa, for the attenuated strains, and my appreciation of advice and assistance which have been so generously given. My thanks are also due to Professor Belfonti for the supply of " Carbozoo " and certain literature.

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Abstracts of Current Literature

The Prevention of Anæmia in Suckling Pigs, with Observations on the Blood Picture. By T. HAMILTON, G. HUNT, and W. CARROLL. Contribution from Illinois Agricultural Experiment Station.

THIS reprint, in pamphlet form, is taken from *The Journal of Agricultural Research*, Washington. The authors refer to the prevalence of anæmia in suckling pigs under present-day management of swine, and the losses occasioned by it. Braasch reported a high death-rate from this malady of suckling pigs in Schleswig-Holstein as far back as 1890. McGowan and Crichton recognised the disease in England prior to 1923, and in 1924 McGowan produced anæmia in piglings experimentally by confining them indoors without exercise, and with access only to the iron-poor diet of the sows.

In 1927, Doyle, Mathews and Whiting reported the results of three years' work on anæmia in swine at the Purdue station :—Twenty-five milligrams of iron is the daily dose which has been successfully used to combat the ailment. The Purdue workers found that anæmia was about four times as prevalent and the death-rate nearly four times as high among pigs kept indoors as among those kept outdoors on concrete. Irradiation with the carbon-arc lamp on pigs confined in a central hog house were little if any better off than the group confined indoors without irradiation. Schofield found that pigs in Canada raised outdoors were resistant to the disease. Hart and his associates point out the fact that anæmia

is common in pigs only between the ages of three and six weeks. If pigs fail to contract the disease before they start eating solid food, they rarely become anæmic. Beneficial effect was always observed when the little pigs had access to soil, pasture or cinders out of doors, or when a ration of blue grass was given to them in the pens. Doyle says that soil alone placed in the pens prevents the disease, although the hæmoglobin content of the blood of these pigs is not so high as when blue-grass sod is used. Willman and McCay, at Cornell, found that drenching the pigs twice a week with a solution of ferrous sulphate, and then placing 0.1 per cent. of the salt in a palatable grain mixture accessible to the little pigs after they were fourteen days of age, entirely prevented the disease. The investigations of the authors of this pamphlet were conducted on five groups of pigs. They found that brushing the sows' udders three times daily, from farrowing up to eight weeks, with the following solution, cured the anæmia of sucklings. The solution used contained approximately 25 mg. of iron and 5 mg. of copper per c.c., and was made as follows: 19.64 grammes of hydrated copper sulphate, and 89.51 grammes of anhydrous ferric sulphate were dissolved in 500 c.c. of water, and this mixture was then added to an equal volume of corn syrup. It was found that putting cinders in the pens helped to produce good results, and that exercise in itself is not a factor in the development or prevention of anæmia in pigs. Post-mortem showed a greatly enlarged heart and a pleural cavity filled with fluid. Briefly, the authors found that nutritional or milk anæmia in suckling pigs is more prevalent in February and March litters than in those farrowed in the late spring. It is not only more prevalent in February and March, but more severe, with a higher death-rate. Brushing the udders of the sows with a dilute aqueous solution containing iron, copper and syrup, or iron and syrup, or *iron alone*, one or more times daily, from farrowing until weaning time, completely prevents anæmia in suckling pigs. The udder treatment for the first four weeks, followed by allowing the little pigs access to a palatable feed mixture containing 0.1 per cent. of added ferric sulphate, is an alternative method which seems to be just as efficient as is the udder treatment for the entire eight weeks.

Nutritional or milk anæmia is an important factor in the mortality of pigs before weaning time. Of 86 pigs having a blood hæmoglobin concentration of 3.5 grammes or less per 100 c.c., 24 per cent. died. Of 109 pigs whose blood hæmoglobin concentration did not reach anæmic levels, only one pig was lost; and whether or not this pig died, was eaten, or strayed away, is not known.

The Active Immunisation of Sheep Against Infectious Entero-toxæmia (Braxy-like Disease) by means of B. ovitoxicus Anaculture. By H. W. BENNETTS: *Journal of the Council for Scientific and Industrial Research* (Australia), Vol. 6, No. 2, p. 92.

THIS article is an account of the results obtained in the field by vaccinating sheep with *B. ovitoxicus* anaculture as a preventive of entero-toxæmia, and of

the results obtained in the laboratory by using other antigens. Bennetts has published in full accounts of his work in connection with this disease, and has shown very clearly that a type of *B. welchii*, which he named *B. ovitoxicus*, found in the intestine of affected lambs is the cause of the condition.

Preparation of the Vaccine.—Bottles containing horse-muscle broth with meat is inoculated with the breast muscle of pigeons which have died as a result of being infected with *B. ovitoxicus*. Glucose (1 per cent.) is added, and incubation is carried on at 37° C. for forty-eight hours. The culture is now filtered through muslin, and 0.5 to 0.7 per cent. formalin is added, and allowed to act till 10 c.c. of the culture is no longer lethal to a guinea-pig (intramuscular injection). This usually takes about fourteen days.

Field Results.

In 1931, 9,679 sheep on twenty-one farms were inoculated with vaccine, only half the animals on each farm being treated, the others being reserved as controls. Two doses, 5 c.c. then 10 c.c., were injected, while younger sheep received half those doses. Except for a passing lameness, no ill-effects were noted. Results were obtained from eight farms only. (On the other farms no disease appeared even among the controls.) Of the 4,538 vaccinated sheep on the eight farms, 22, or 0.48 per cent., died ; while of the 5,718 controls, the death-rate was 151, or 2.64 per cent., *i.e.*, " the mortality-rate was reduced to only 18 per cent. of its former value." It is interesting to note that in the field experiments with vaccine (anaculture) made from a certain strain (T2) the results were not so favourable as above. Bennetts, discussing this result, says : " This was not entirely unexpected, as on titration of cultures from which this vaccine was made, it was found that the toxin content was comparatively low . . . and subsequent laboratory experiments also indicate that the T2 strain was of low antigenic value, and it was not used further for vaccine production."

During 1932 the Commonwealth Serum Laboratory supplied most of the vaccine used. The doses of vaccine used were reduced to 3 and 7 c.c. for adult sheep, and 2 and 5 c.c. for young sheep. True controls could only be kept in two instances. In all, 16,589 sheep on twenty-six farms were treated, both inoculations being given by at least three weeks before lambing. No ill effects were reported, even when lambs two to three weeks old were inoculated. On eight of the farms only was there any death-rate. On these farms 7,743 sheep had been treated, and only thirty deaths occurred, *i.e.*, 0.39 per cent. On the two farms where controls were kept the result was : of 913 treated sheep, 5, or 0.55 per cent., died, while the death-rate among the 1,109 untreated sheep was 41, or 3.61 per cent., *i.e.*, the inoculation lowered the mortality-rate to approximately 15 per cent. of its former value.

A further experience of the use of vaccine is recorded, *viz.*, the immunisation of lambs fattening on peas. It appears that in Western Australia lambs are kept during the summer months on crops of shed field peas, and that scouring frequently results with death, which Bennetts has shown to be true entero-

XUM

toxæmia. The average death-rate on one farm was 5 per cent. In 1932, 503 lambs on this farm were vaccinated with 2 and 5 c.c. anaculture, while 95 were left as controls. The result was a death-rate of 3.15 per cent. among the controls, while the treated sheep all remained healthy, the owner reporting " that a feature of the lambs this year was the freedom from scouring, only a few showing it," and, further, that " it was remarkable how few of the uninoculated lambs were suitable for inclusion in the first draught for market." The results of these two seasons' observations are that adult sheep and lambs can be successfully immunised against entero-toxæmia by the use of *B. ovitoxicus* anaculture.

Laboratory Experiments with Modified Vaccines.

The second part of the paper deals with experiments to produce a better product, or one which would give equally good results with a smaller dosage. Two lines were tried :

(1) *Alum Toxoid.*—Bennetts attempted to immunise guinea-pigs and to hyper-immunise rabbits with antigen prepared by the technique detailed by Glenny, *viz.*, the precipitation of toxoid with potash alum, but " the results of a series of experiments indicated that in laboratory animals the response to these products was not superior to that obtained with anaculture. It was concluded that the dead bacteria in the vaccine promoted sufficient local reaction to prevent the too rapid absorption and elimination of the anatoxin, as well as the bacteria themselves possibly acting as antigens."

(2) *Lamb Dysentery Bacillus Anaculture.*—An account is given of the *B. welchii* group of organisms, and special reference is made to the work of Wilsdon in classifying this group. It seems probable that the organism described by Wilsdon as " D " and Bennett's *B. ovitoxicus* are very closely related. A reference is also made to the publication of Dalling, in which it is suggested that the *B. ovitoxicus* and *B. poludis* are identical. It is further recorded that Dr. L. B. Bull, Adelaide Hospital, suggested " an antigenic relationship of the members of the *B. welchii* group, which was somewhat similar to that later reported by Wilsdon." Dr. Bull thought, further, that " the lamb dysentery bacillus toxin might contain the most evenly-balanced mixture of antigenic components." For this reason, and the fact that the latter organism produces a more potent toxin than *B. paludis*, Bennetts decided to carry out immunisation in laboratory animals with lamb dysentery anaculture, and test them with *B. ovitoxicus* toxin. Guinea-pigs were injected twice with the anaculture, and tested later by intra-muscular injection of toxin. The test was controlled in guinea-pigs injected with *B. ovitoxicus* anaculture, and showed that while *B. ovitoxicus* anaculture protected the animals against at least one M.L.D. of *B. ovitoxicus* toxin, those receiving lamb dysentery anaculture had acquired no immunity whatever. This work was discontinued. It is proposed to carry out further field and laboratory experiments, especially with a view to ascertaining the duration of immunity following routine vaccination.

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Summary.

(1) Sheep may be actively immunised against entero-toxæmia by means of *B. ovitoxicus* anaculture. Two inoculations with three weeks' interval are recommended. During the 1931 and 1932 seasons the mortality-rate in controlled flocks was reduced by 85 per cent. as a result of inoculation. (2) A small experiment indicated that lambs fattened on shed peas during the summer months can be satisfactorily immunised by this method. (3) Lamb-dysentery bacillus anaculture did not immunise guinea-pigs against *B. ovitoxicus* toxin, although a response was obtained with *B. ovitoxicus* anaculture. (4) *B. ovitoxicus* "alum toxoid," tested on guinea-pigs, did not possess any greater antigenic efficiency than *B. ovitoxicus* anaculture.

Clinical Articles

Deaths in Poultry Occasioned by Bee Stings.

By H. P. HAMILTON, B.V.Sc. (Toronto),
Poultry Research Laboratory, Goring-on-Thames.

On July 11th, 1933, I was asked for advice by a lady who keeps a few fowls in her garden for a hobby and egg supply to the house. I was informed that ten 1932 pullets were perfectly healthy at 5 o'clock, and at 9 o'clock, when I was called in, four of them had died, and the others were on the point of death. I closely examined them and found that the cause of death was bee stings, and from one of the birds that had died I extracted 167 stings. The heads were swollen as a result of the stings, and I am sending you this note in case it may be of interest to practitioners engaged on poultry work. There were several hives, not belonging to the owner of the birds, just over the hedge, and doubtless the bees in question came from these hives, as the owner of them had been working among his bees during the afternoon.

Correspondence

THE EFFECT OF A CALCIUM-DEFICIENT DIET ON PREGNANT EWES.

To the Editor of The Veterinary Journal.

Sir,—If the ill-health shown by the four ewes of the basal group in the experiment discussed by Fraser, Godden and Thompson (Vet. Journal, Vol. 89, No. 9) is due to pregnancy disease and not to dead lambs *in utero*, etc., the results confirm our original findings of 1931, which have since been confirmed by Hopkirk and Gill, Wallaceville Veterinary Laboratory, that under-feeding alone precipitates the disease in susceptible subjects and that exercise is of no preventive value.* However,

* Leslie, A.: *Australian Vety. Jnl.*, Feb., 1933.

the authors associate the onset of the syndrome with a lime-deficient diet causing a serum-calcium shortage. As the post-mortem findings on ewes 38 and 39 are either different from or not typical of cases of the disease in New Zealand, and the control group was better fed during the susceptible pregnancy disease period than those of the basal group, the separation of the ætiological effects of a low serum calcium from those of under-nutrition, which were operating at the same time, becomes less easy. In fact if these deductions are correct, they tend to invalidate the interpretation of the results by the authors, that a lime-deficient diet may be a factor in the syndrome of pregnancy disease. Had the ewes on the basal group continued to consume equal quantities of food to those in the control group, the significance of a calcium-deficient diet in the ætiology of this disease would have been established. In ten typical New Zealand pregnancy disease cases the serum-calcium estimations ranged from eight to normal, and calcium administrations orally, subcutaneously and intravenously to over twenty cases, produced no apparent curative benefit. For these reasons and the fact that underfeeding can precipitate the disease in susceptible flocks, I am inclined to the view that had the serum-calcium values been raised in the basal group during the susceptible disease period, and *the animals underfed*, ill-health similar to that described would have been produced if the subjects were of the susceptible age and prolificacy.

ALLAN LESLIE.
Canterbury Agricultural College (*Veterinary Department*).

News

THE INTERNATIONAL HORSE SHOW. LORD MAYOR CHRISTENS MONSTER NOSEBAG FOR THE ROYAL VETERINARY COLLEGE WITH A THOUSAND FARTHINGS.

AFTER attending the opening afternoon performance of the International Horse Show on Thursday, June 21st, the Lord Mayor and Lady Mayoress, the Sheriffs and the City Marshal were conducted to the stables by Sir Frederick Hobday, Principal and Dean of the Royal Veterinary College.

Much delight was given to the younger members of the party when the Lord Mayor's little grand-daughter, Miss Jane Alexander, was placed in the saddle and taken round the pony section.

The party then crossed over to see the War veteran chargers, whose owners had sent them there to support the Show. These veterans range in age from twenty-five to thirty years, and most of them have been wounded in the service of their country. And here outside Peter's box—Peter being a veteran of twenty-seven, and owned by Mr. G. A. Tomlin, M.R.C.V.S., of Woodbridge, Suffolk—was a giant nosebag, 5 ft. by 4 ft. 6in., on which appeared the following legend :

> " *For the Royal Veterinary College.*
> *The World's Largest Nosebag.*
> *Please help to fill it with golden hay.*
> *Presented to Sir Frederick Hobday by Geo. Parker & Sons.*"

After smilingly reading the inscription, the Lord Mayor, Sir Charles Collett, wished the cause " Good Luck," and christened the bag by placing in one of the money slots a thousand farthings.

Turning to Sir Frederick, the Lord Mayor said : " I wish you Godspeed in your efforts on behalf of your college, and the cause of sick animals. May you soon get the quarter of a million pounds that you still need." The Lady Mayoress was also most interested in this novel collecting bag, and said : " What a really wonderful idea. I wish you, Sir Frederick, every success. It is splendid." This monster nosebag is to be used for collecting purposes for the Royal Veterinary College at horse shows throughout the country.

Personal

MR. J. W. BURGESS, M.R.C.V.S., has been appointed Assistant to the Professor of Surgery and Obstetrics in the Royal (Dick) Veterinary College, Edinburgh.

MR. J. D. MCLAUCHLAN, M.R.C.V.S., D.V.S.M., has been appointed assistant in the Veterinary Department of the City of Stirling.

MR. J. W. PROCTOR, M.R.C.V.S., has been appointed part-time Veterinary Inspector to the Corporation of York in the place of Mr. W. Pollock, F.R.C.V.S., who resigned.

THE Felixstowe Urban District Council have decided to ask the East Suffolk County Council to appoint a whole-time Veterinary Inspector in the Department of Public Health in order to more effectually control the dairy herds and the milk supply.

THE Devonshire County Council have appointed Mr. D. S. Barbour, M.R.C.V.S., the late Chief Assistant to the Ayrshire County Council, as Chief Veterinary Inspector to the County of Devon.

CAPT. GEORGE F. SCOTT ELLIOTT, B.A. Cantab., B.Sc. Edin., formerly Professor of Botany in the Glasgow Veterinary School, recently died at the age of seventy-two. Professor Elliott was a noted scientist and traveller, and had accompanied several Government expeditions as botanist, and had written a number of books on this subject.

PROFESSOR SHARE JONES has had conferred upon him the Honorary Doctorate of the Veterinary Faculty of the University of Berne. The grounds upon which it was given being stated " that he had changed and enlarged the conception of veterinary science, created for it new opportunities for service, and advanced the cause of veterinary education."

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